JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXXII WASHINGTON, D. C., APRIL 15, 1925 No. 8

CYTOLOGICAL STUDIES OF FORMS 9, 21, AND 27 OF PUCCINIA GRAMINIS TRITICI ON KHAPLI EMMER[1]

By RUTH F. ALLEN[2]

Associate Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The physiologic forms of stem rust of wheat are similar morphologically. Their differing range of infective power is due to physiological differences. In the ultimate analysis, of course, those differences are chemical in nature. The study of variations in the metabolism of these fungi, in the nature and amount of their secretions, and in their reaction to the substances present in the host cells or produced there as the result of fungous activities, are problems for the biochemist. There are serious obstacles, however, in the way of a chemical analysis of an obligate parasite, for one can not obtain an extract of the organism free of host substances. Pending such an analysis, further information as to the nature and behavior of these forms of the fungus and their effects on various hosts may be obtained by genetical and microscopical studies. The present paper is the third of a series of cytological studies on this subject.

The literature of cereal rusts has grown rapidly. Earlier papers presenting cytological studies of cereal rusts, genetical studies of rust resistance, and evidence of physiologic forms in cereal rusts, have been reviewed frequently and need not be repeated here. There have been several recent additions to this literature.

Stimulated by the discovery of physiologic forms of stem rust on wheat (*20*)[3] and the rapid development of the work of determining the number, distribution, and means of identification of these forms (*21*), similar work has been undertaken on other cereal rusts. The existence of physiologic forms has been reported by Haerner (*6*); by Stakman, Levine, and Bailey (*22*) in *Puccinia graminis avenae* Erikss. and Henn.; by Levine and Stakman (*13*) in *Puccinia graminis secalis* Erikss. and Henn.; by Mains and Jackson (*15*) in *Puccinia triticina* Erikss.; and by Melhus, Dietz, and Willey (*17*) in *Puccinia coronata* Cda.; while Hungerford and Owens (*7*) find "* * * some indication also that there are two or more strains or specialized forms of *Puccinia glumarum tritici* Erikss."

[1] Received for publication Aug. 12, 1925; issued March, 1926. Cooperative investigations between the Agricultural Experiment Station of the University of California and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[2] The writer is indebted to C. R. Ball, senior agronomist in charge, and H. B. Humphrey, senior pathologist, of the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, for suggestions and for reading of this manuscript, and to the Divisions of Genetics and Agronomy of the University of California for courtesies extended during the work.

[3] Reference is made by number (italic) to "Literature cited," p. 724.

Miss Newton (*18*), in 1922, made a wheat-rust survey of a large area in Canada, and found there 14 of the 37 physiologic forms of wheat stem rust which are found in the United States. The paper includes also the results of a cytological study of the entry and early development of form 17 in Marquis (a susceptible host) and Kanred (resistant).

Levine (*12*), in 1923, made a statistical study of spore sizes in the main subdivisions of *Puccinia graminis*. Aeciospores, urediniospores, and teliospores of *Puccinia graminis tritici, secalis, avenae, agrostis,* and *phleipratensis* were measured, and found to show differences in size, and, to a lesser extent, in form. These differences are constant when conditions are favorable. Unfavorable conditions, such as excessive heat, poor light, drought, or uncongenial host, reduce the size of the spores, reduction in length being greater than reduction in width.

Miss Hurd (*8*), in 1923, found no correlation between hydrogen-ion concentration of wheat varieties and their resistance to disease. Khapli emmer and Little Club wheat, for example, which represent the extremes of resistance and susceptibility to stem rust, have about the same hydrogen-ion concentration.

Hursh (*9*), in 1924, showed that morphological characters of wheat varieties, while not affecting the fundamental protoplasmic resistance of the plant to stem rust, might affect the amount of rust which could grow upon it. Entrance of the fungus through the stoma was studied, the facts noted that many stomata remain closed, and many fungi fail to enter, and the conclusion reached that (*9, p. 389*) "before the results of different experiments can be compared, it will be necessary to control all the factors which influence the movement of stomata during the incubation period." On stems, the rust grows only in collenchyma, and can not penetrate the sclerenchyma. If the collenchyma occurs in narrow, isolated strands surrounded by sclerenchyma, the spread of the mycelium is sharply limited.

Stakman and Aamodt (*23, p. 376, 375*), in 1924, published results of experiments on the effect of fertilizers on stem rust of wheat. They find that "while the direct effect of fertilizers on the susceptibility of wheat to stem rust under field conditions is slight, the indirect effect on rust and the direct effect on yield may be very great," and that "nitrogen apparently may predispose plants [to rust] by increasing succulence, density of stand and lodging, by delaying maturity, and by decreasing yields on account of these factors, and sometimes by premature ripening in hot, dry weather."

Mains and Leighty (*16*), in a study of leaf rust, *Puccinia dispersa* Erikss., on 68 selections and varieties of rye, found all gradations from high susceptibility to complete immunity. No variety was uniformly resistant, but all contained a few resistant plants. The initial work in crossing indicated that resistance is dominant, and that there may be complicating factors.

Sax (*19*), in 1923, in a genetical study of a cross between *Triticum vulgare* (with 21 chromosomes) and *T. durum* (with 14 chromosomes), finds in the F_3 generation that most of the segregates have either 21 or 14 chromosomes and that those with an intermediate chromosome number tend to be eliminated. As those with 21 chromosomes have the morphologic character and susceptibility to rust of the *T. vulgare* parent, and those with 14 have the characters and rust resistance of the *T. durum* parent, the writer concludes that there is little

likelihood of combining the desirable characters of *T. vulgare* wheats with the rust resistance of the *T. durum* group.

Harrington and Aamodt (*4*) made crosses between durum wheats, two of which, Mindum and Pentad, reacted reciprocally to forms 1 and 34 of stem rust. A few of the F_3 families showed marked resistance to both forms of the rust. In some cases the resulting rust resistance can be accounted for fairly well by one main factor; others were less clean-cut.

Aamodt (*1*), in 1923, published an account of the progeny of a cross between Marquis, a spring wheat susceptible to stem rust, and Kanred, a winter wheat immune from many of the physiologic forms of stem rust. F_3 families were obtained that were homozygous for spring habit of growth, and immune from attack by several of the forms of rust from which the Kanred parent is immune. Rust resistance is inherited as a single genetic factor, and immunity is dominant over susceptibility.

Hayes and Aamodt (*5*) studied rust resistance in a cross between Kota (susceptible to form 19 and immune from form 27) and Marquis (resistant to form 19 and moderately resistant to form 27). In the F_3 generation field tests with form 19 gave complex results that could not be explained on the basis of a single factor, and tests with form 27 "could be quite satisfactorily explained on the basis of two independently inherited factors for immunity and resistance contained in the Kota and Marquis parents, respectively, each factor being allelomorphic and dominant to a factor for susceptibility."

A few F_3 families combined the rust resistance of the parents to the two forms of rust, and in a field test with several forms of rust a few were as resistant as Kota. Greenhouse tests of seedlings were considered unsatisfactory as an index of rust resistance under field conditions.

In former studies (*2*, *3*), seedlings of Kanred were inoculated with the physiologic forms of *Puccinia graminis tritici* Erikss. and Henn., numbered 3 and 19, and the infected material was studied cytologically. As Kanred is immune from form 19 and susceptible to form 3, the two cytological pictures were sharply contrasted—so sharply, in fact, that comparisons were difficult.

A more exacting test of physiological differences between forms of stem rust would be a study of several rusts which produced the same grade of infection on a given host. In such material differences could be studied, measured, and compared, and some insight into the nature of those differences might be gained. For this purpose, a comparative cytological study has been made of physiologic forms 9, 21, and 27 on Khapli emmer.

Khapli emmer (*Triticum dicoccum* Schr.) is unique among wheat allies in being highly resistant to all the known physiologic forms of wheat stem rust. One variety of wheat may successfully combat certain forms of the rust, and a second may be immune from others. Each of the two hosts may be susceptible to the forms of the rust that the other resists. Khapli alone resists them all. The infections would grade (on a scale of 0 to 4, on which 0 represents immunity and 4 complete susceptibility) between 0+ and 1+.

MATERIAL AND METHODS

Seed of Khapli emmer (C. I. 4013) and the other differential hosts used at the Minnesota Agricultural Experiment Station for determining physiologic forms or races of stem rust, and viable spores of several forms of stem rust, were sent from the Minnesota station by M. N. Levine in the summer of 1922. Seedlings of Little Club wheat were grown in the greenhouse, and a single-spore culture of each rust was started. These were kept under bell jars for 48 hours and then isolated in cheesecloth cages in the greenhouse. From each of these pure cultures, after an increase generation, the Khapli seedlings intended for study were inoculated, and a set of differential hosts was inoculated to test the identity of the rust. Infected Khapli material was fixed daily from the third to the tenth day and at longer intervals from then on to the eighteenth day. Some of this material was kept at room temperature during fixation, but the greater part of it was placed in a cold-storage chamber at 45° F. It was washed, dehydrated, and infiltrated with paraffin, and slides were prepared by the usual cytological methods.

INVESTIGATIONS

MACROSCOPIC OBSERVATIONS ON LIVING MATERIAL

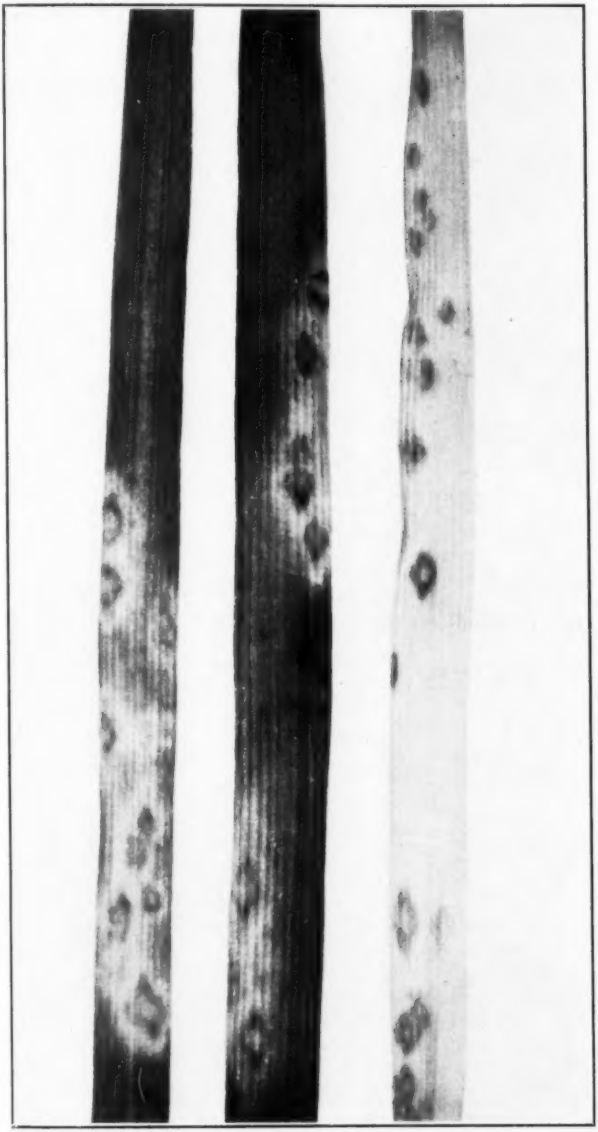
Differences in the three rusts on Khapli emmer can be detected even in the living material. Daily observations show (Table I) that the first visible discoloration of the leaf at points where infections have started appears on the fourth day in case of forms 9 and 21, and on the fifth or sixth day when form 27 is used.

In form 9 there is a slow, somewhat uneven rate of increase in the size of the fleck, the maximum diameter being 2 or, more rarely, 3 mm. In form 21 the spot increases to 1 mm. by the seventh day, remains at that size for several days, then slowly increases up to 2 mm. In form 27 the discoloration is later in starting, but once started it increases rapidly to 1½ mm., then remains at that size for some time. Between the twelfth and the fourteenth days an outer halo of yellowed tissue appears, separated from the inner spot by a zone of green tissue (pl. 1). Simultaneously, the central fleck decreases in diameter, for its outer tissues regain chlorophyll. In a few cases the whole central fleck turns green. The final size of the whole, in form 27, is 3 to 7 mm. in diameter, which is more than twice the size of flecks from either form 9 or form 21.

The infected seedling leaf dies earlier than the uninfected, and eaves bearing numerous infections die sooner than those with few. In case of the first two forms, 9 and 21, the infected leaves are dead by the eighteenth day. In case of form 27, some are still green at that time. Moreover, as the leaf dies the infected areas of form 27 (pl. 1) retain chlorophyll after the rest of the leaf has yellowed.

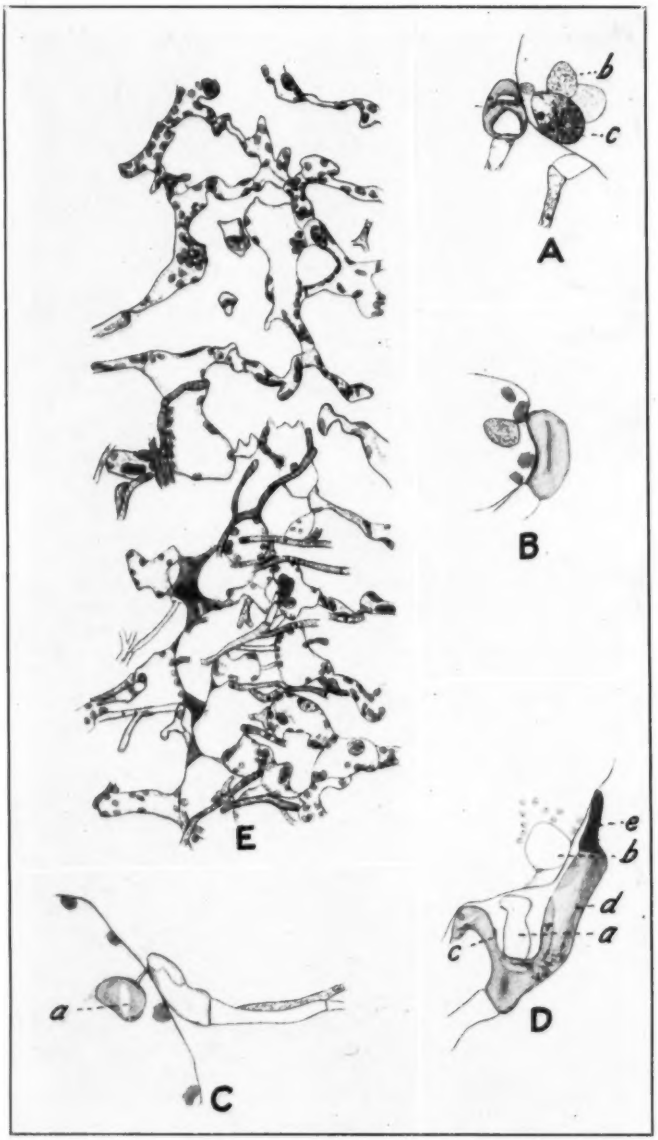
The majority of the infections yield no spores. An occasional minute uredinium forms on flecks from forms 9 and 21. No spores have been noted on flecks from form 27.

The dimensions of these flecks and the number of spores produced might have been greater if these seedlings had been grown in the field instead of in the subdued light within cheesecloth cages in the



Form 27 of *Puccinia graminis tritici* on Khapli emmer

Photograph of seedling leaves bearing infections 19 days old. Surrounding the central pale area in each infection is a zone of green tissue, and surrounding this is a secondary pale region. As the leaf dies, the green zone retains chlorophyll when the rest of the leaf is yellow. × 3



Form 9 of *Puccinia graminis tritici* on Khapli emmer

A.—Ten-day infection. Wall of mother cell *a* irregularly swollen, leaving two separate portions of the lumen. Haustorium *b* severed from mother cell by host nucleus *c*. × 1130

B.—Ten-day infection. Haustorium mother-cell wall swollen, obliterating cavity. × 1130

C.—Ten-day infection. Small portion of mother-cell wall swollen. Haustorium *a* also modified. × 1130

D.—Twelve-day infection. Old mother cell *a* and haustorium *b* nearly digested. The hypha below and its lateral branches *c* and *d* have walls swollen. Young mother cell *e* collapsed. × 1130

E.—Five-day infection. Longitudinal section through the margin of infection and the tissues beyond. Very little impoverishment and no plasmolysis of tissues beyond the fungus. × 333

greenhouse. But, since the three rusts were grown at the same time under parallel conditions, the relative differences should be trustworthy.

TABLE I.—*Macroscopic observations on living material of Puccinia graminis tritici forms 9, 21, and 27, on Khapli emmer*

Days after inoculation	Form 9		Form 21		Form 27	
	Diameter of fleck	Description	Diameter of fleck	Description	Diameter of fleck	Description
4	----------	Vague, infected area, a shade lighter in color.	----------	Vague, minute spots.	----------	No visible change.
5	¼ mm. or less.	Numerous pale green flecks.	¼ mm. or less.	Numerous distinct whitish flecks.	----------	Doubtful, vague if any.
6	¼ to 1 mm.	Outline vague; margin fading.	½ mm.	Distinct flecks	½ mm. or less.	Whitish, distinct.
7	½ to 1 mm.	Fleck white, distinct.	1 mm	Pale green, circular, distinct.	1½ mm. or less.	Whitish, irregular, sometimes rhomboidal.
9	1 to 1½ mm.	Fleck very white; faint "green island" at center; no spores noted.	About 1 mm.	Circular white fleck; few minute uredinia.	1½ mm	No spores; flecks whitish, sometimes with faint green center.
12	2 mm. or less.	Circular dead area; few or no spores.	1 mm	Circular dead area; very few bear spores.	1½ mm	No spores; fleck circular, whitish.
14	2 mm	Flecks have fading margin and dead white center; very few bear spores; leaves dying.	2 mm	Leaves dying; strong fleck white in center; very few bear spores.	3 mm	No spores; central fleck decreasing in size; secondary halo of yellow separated from central fleck by green tissue.
18	2 to 3 mm.	Leaves nearly all dead and dry; very few spores; fleck dead and white.	2 mm	Leaves nearly all dead; fleck very white and dead; very few spores.	3 to 7 mm.; average 4½.	No spores; concentric zonation still conspicuous (pl. 1); central discolored area averages 1.1 mm., which is less than it was in younger material; some of infected leaves living, some dead; infected areas retain green color even when remainder of leaf is yellowed (pl. 1).

ENTRY OF THE FUNGUS AND THE EFFECT ON THE STOMATA

In no case studied do all of the fungi enter the host. In Khapli emmer also, some of the fungi are excluded. Fifty-two per cent (Table II) of form 9 entered the host, 42 per cent of form 21, and 21 per cent of form 27.

In previous studies it has been noted that a stoma through which a fungus enters the host may undergo changes. The walls of the guard cells become altered chemically and lose affinity for ordinary stains. This change begins at the surface of contact with the fungus and spreads to the cell contents and farther walls of the cell. In extreme cases the guard cells are killed and adjoining walls of epidermal cells are affected.

This damage to guard cells is very slight with form 9 on Khapli emmer, moderate with form 21, and extreme with 27 (Table II). As the host is the same and the three lots of material were subjected to the same conditions, this marked difference in the effect on the guard cells must mean a difference in the amount or the concentration of the secretion by the appressoria.

TABLE II.—*Comparison of entrance of physiologic forms 9, 21, and 27 of wheat stem rust, and their effects on the stomata of Khapli emmer*

Form 9	Form 21	Form 27
Entries: 26 out of 50 fungi enter, or 52 per cent, in material fixed 3 to 10 days after inoculation	Entries: 36 out of 86 fungi, or 42 per cent, enter in material fixed 4 to 7 days after inoculation	Entries: 16 out of 75 fungi, or 21 per cent, enter in material taken 5 to 7 days after inoculation
Of the stomata occupied by these 50 fungi, 45 (or 90 per cent) of the guard cells remained normal. 5 (or 10 per cent) of guard cells were slightly injured.	Of the 86 occupied stomata, 46 (or 53.5 per cent) of the guard cells remained normal. 16 (or 18.6 per cent) of the guard cells were slightly injured. 14 (or 16.3 per cent) of the guard cells with central shaft of cell killed. 10 (or 11.6 per cent) of the guard cells were killed.	Of the 75 occupied stomata, 17 (or 22.6 per cent) of the guard cells were normal. 14 (or 18.7 per cent) of the guard cells were slightly injured. 15 (or 20 per cent) of the guard cells with central part were killed. 20 (or 26.7 per cent) of the guard cells were dead. 9 (or 12 per cent) of the guard cells were dead and adjoining cells injured.

GROWTH AND PROGRESS OF THE FUNGUS

Cytological study of these physiologic forms of the rust on Khapli emmer shows that damage done to the tissues frequently is discernible under the microscope for a considerable period before it becomes visible macroscopically.

Moreover, a brief study suffices to show that the size of the discolored area or fleck, as seen in the living leaf, is not an accurate index of the diameter of the area containing mycelium. At one time the fungus has spread farther than the visible damage, at another the areas of spread of the fungus and of the visible damage are about the same size, and at still another the damaged area is considerably larger than that occupied by mycelium. These differences in the relative sizes of the flecks and mycelial areas vary with age, and each of the three rust strains studied has its own characteristic sequence and proportions.

INFECTIONS BY FORM 9

Infections by form 9 five days old have spread nearly as far as they would have spread if the rust and host had been congenial, but the mycelium is less dense. The diameter of the mycelial areas ranges from 330 μ to 400 μ. As the flecks visible in the living material at five days are 250 μ or less in diameter, it is evident that the discolored area covers only the central older part of the mycelium. There is, however, a hint of coming change out beyond the fungus. Plastids there are slightly reduced in size. Plate 2, E, represents the margin of such an infection and the tissues beyond it.

On the sixth day a zone of leaf tissue, which may be several hundred microns wide beyond the fungus, shows distinct impoverishment. Moreover, this effect on the outlying regions soon becomes much more extreme than it is in the tissues occupied by the fungus, so that this central area becomes a "green island" of living cells in the center of an area of dying or dead tissue. In Plate 3, A, is shown a narrow strip through the leaf at the center of an eight-day infection, and in Plate 3, B, is a similar strip through the dead area that surrounds it. In other cases the outer zone is still living, although seriously injured. Table III shows plastid sizes on the different days after inoculation. This ring of dead or dying tissue

TABLE III.—*Size of plastids on different days after inoculation at center of infection and 750 μ from center, in infections by Puccinia graminis tritici forms 9, 21, and 27, on Khapli emmer. Each measurement represents the average size in microns of 10 plastids*

Days after inoculation	Form 9		Form 21			Form 27	
	Center	750 μ from center	Center	Just beyond fungus	750 μ from center	Center	750 μ from center
5	First infection 3.1×2.3 μ; 2d, 2.8×1.9 μ; 10 to 20 per cent of cells collapsed and dead.	3.2×2.7 μ; normal.	2.9×1.9 μ good many dead and collapsed cells at center.	4.3×1.4 μ; these deformed plastids stain red.	3.3×2.5 μ; normal.	First infection 3.0×2.2 μ; 2d, 3.3×2.5 μ; not more than half a dozen dead cells.	3.3×2.5 μ; normal; (7-day: 3.4×2.0 μ).
8	First infection 2.3×1.3 μ; 2d, 3.7×1.9 μ; not more than 20 per cent of cells in center dead and collapsed.	First infection 0×0 μ; cells dead and empty; not collapsed; 2d infection 2.2× 8 μ; some of cells contain no plastids.	2.6×1.7 μ; 40 or 50 per cent of cells collapsed and dead.	4.9×1.1 μ; plastids deformed and stain with saffranin.	3.2×2.0 μ; fungus has not reached this point.	2.7×1.6 μ; few cells dead and collapsed; mere hint of red-stained tissue at sides of infection.	2.5×1.3 μ; fungus has just reached this point.
10	2.0×1.5 μ; central area nearly all dead.	2.8×1.1 μ; fungus past this point.	0×0 μ; dead; only a few traces of plastids left.	Mere trace of deformed plastids.	2.2×1.3 μ; nearly all dead; fungus has passed this point.	2.9×1.8 μ; relatively few cells dead and collapsed.	2.8×1.7 μ; cells impoverished, but very few dead and collapsed.
14			0×0 μ; dead; only occasional trace of plastids.		0×0 μ; dead; whole leaf dying in some cases.	2.4×1.3 μ; about 50 per cent of cells dead.	2.9×2.0 μ; not more than 10 per cent of cells dead and collapsed.
18	0×0 μ; dead.	0×0 μ; dead; rarely a cell shows trace of plastids.	0×0 μ; rare cell still shows trace of plastids.		0×0 μ; occasional living cell.	2.1×1.3 μ; about 60 per cent dead and collapsed.	2.4×1.4 μ; some cells empty and clear; not more than 10 per cent of dead collapsed cells.

surrounding an infection looks like an effective barrier to marginal growth of the inclosed fungus. The fungus usually does make its way out, however, if the tissues are not completely killed, although progress through the impoverished area is slow. If these tissues are not too enfeebled, the presence of the fungus stimulates them into a very slight temporary increase in activity. By the time the fungus reaches the green tissues beyond this zone, the leaf as a whole is beginning to die. In 10-day infections, the longitudinal diameter of the mycelial area is sometimes 1,750 μ. The arrival of the fungus in fresh food supplies is not attended by any marked increase in the vigor of its growth, nor does this advance growth of the fungus produce any secondary, impoverished zone beyond its new boundaries. When the death of the leaf checks further growth, the dead white fleck is 2,000 μ, or more rarely 3,000 μ, in diameter.

Infections by Form 21

Form 21 is more uncongenial with Khapli emmer than either form 9 or form 27. Mycelial areas of form 21 five days after inoculation range from 200 μ to 330 μ in diameter, and the affected zones surrounding the mycelia (as seen under the microscope) range from 0 μ to 200 μ in width (pl. 4, D). A day later the larger mycelial areas have exceeded 700 μ and the outer zones surrounding them have more than doubled in width. Not until the seventh day, however, does the extent of this zone become evident macroscopically. From the sixth day to the tenth, there is little or no increase in the size of the fleck. Either the substance which has been diffusing out from the center and causing this damage is no longer being formed, or, if it is still forming, the damaged tissues are too nearly dead to transmit it.

The central tissues usually die rapidly, although occasionally they survive a few days longer. By the sixth day the fungus has started to grow out through the outer dying zone (pl. 5, A), and by the ninth it has ordinarily reached fresh tissues (pl. 5, B). The condition at the center on the tenth day is shown in Plate 6, A. The host cells are dead and have collapsed into little, irregular, strongly staining masses.

At this time (tenth day) the fungus and the area damaged by it are just about equal in extent. No revival of the cells in the area traversed by the fungus has been noted; in fact, they collapse and die rapidly. As the fungus continues a slow marginal growth, the invaded tissues collapse quickly, so that fungus and fleck increase in diameter at the same rate. The final size, when the death of the leaf stops the process, is 2,000 μ or less.

Infections by Form 27

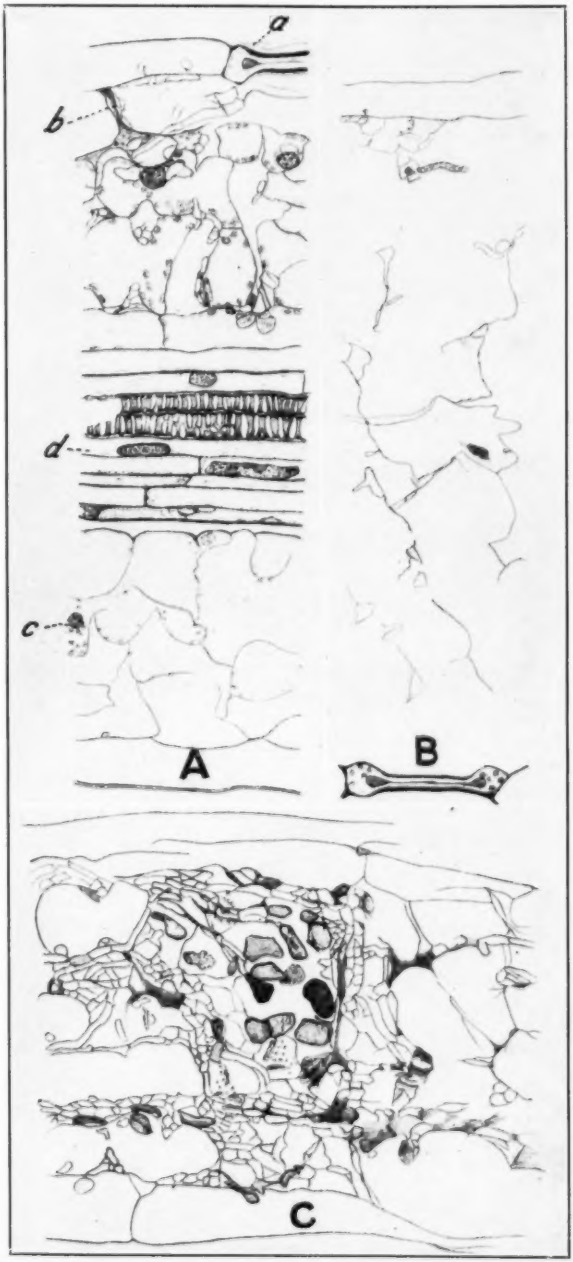
This physiologic form of the stem rust of wheat is the least toxic to Khapli emmer, and the mycelia attain the greatest development. The first cells attacked by the fungus are killed promptly, as in the other

EXPLANATORY LEGEND FOR PLATE 3

A.—Eight-day infection. Narrow strip from one surface of the leaf to the other through the center of an infection. Fungus entered stoma at *a*; dead cell at *b*. Other invaded cells green and living. Tissue beyond fungus, at *c*, impoverished but not collapsed. × 333

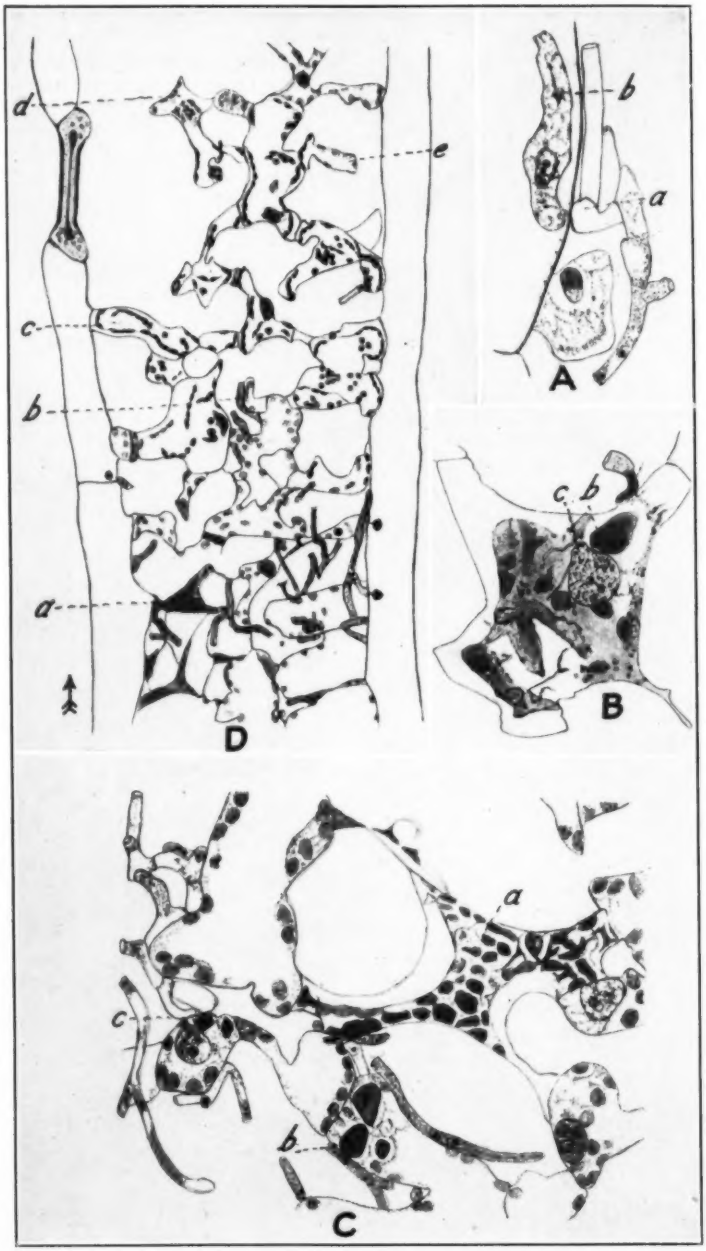
B.—Narrow strip through the dead area surrounding the infection drawn in A. Cell contents and parts of wall digested. × 333

C.—Eighteen-day infection. Section through inclosed pocket in a small uredinium which opens on the surface at another point. Host dead. × 333



Form 9 of *Puccinia graminis tritici* on Khapli emmer

For explanatory legend see page 708



Form 21 of *Puccinia graminis tritici* on Khapli emmer

For explanatory legend see page 709

two cases, but the adjustment between host and rust takes place quickly. The mycelial areas are no larger on the sixth day (500 to 625 μ) than they were in form 21; in fact, they are slightly smaller, but the host is in better condition. Not until the seventh day are there signs of impoverishment beyond the limits of the mycelium. In the cells containing haustoria, plastids average 2.9 by 2.0 μ. At a distance of 450 μ beyond the edge of the mycelium they average 2.5 by 1.7 μ, and 750 μ beyond they are 3.5 by 2.0 μ, or almost normal in size. From this it may be seen that in a zone over half a millimeter wide surrounding the infection the plastids have been rapidly reduced from normal size to dimensions somewhat smaller than those in the infection itself. In other words, the area occupied by the fungus is now greener than the region surrounding it, and we have the "green island" again.

The stolons of the fungus push out through this damaged region. As may be seen from the drawing (pl. 7, B) which represents the condition on the ninth day, the host cells are still living. When the hyphae in passing stop to form haustoria, the invaded cells often are stimulated, the cell contents become richer and the plastids larger. In marked contrast to the resultant of the balance of forces in the other two forms, part or all of this outer zone regains a green color, and more rarely (pl. 1) the central area also turns greener. Table III shows the sequence in the changes in plastid size at the different periods.

In spite of these apparently favorable conditions, the fungus does not gain in vigor nor can it store up reserves or produce spores. Its whole strength is devoted to marginal growth, which continues until it reaches fresh green tissues beyond the fleck.

Development of the fungus in the green outer tissue weakens but does not destroy it. Between the twelfth and fourteenth days a secondary impoverished zone with reduced plastids appears out beyond the fungus. The diameter of the whole is 3 to 7 mm.

The condition of the host within the scope of the mycelium in an 18-day infection is shown in Plate 8: *A* represents a narrow strip through the leaf at the center; *B*, midway; and *C*, at the margin of the mycelium. In this infection the center is pale. Close inspection, however, shows that remarkably few of the cells collapsed and that some still contain a nucleus and minute plastids. When, as commonly happens, the infected leaf dies prematurely and the uninfected parts turn yellow, the areas occupied by mycelium still remain green and hold their chlorophyll more tenaciously than healthy tissues.

FIRST ATTACKS OF THE FUNGUS AND THE LATER ADJUSTMENT

On the third day following leaf inoculation, small mycelia may be found in the intercellular spaces of the mesophyll tissue, and the fungi have made their first attempts to establish food relations with

EXPLANATORY LEGEND FOR PLATE 4

A.—Four-day infection. Large haustorium *b* (26 μ long) in an epidermal cell. × 1130

B.—Four-day infection. Dying mosophyll cell. Collapsed mother cell at *a*. Host nucleus *c* beside the dead haustorium *b*. Plasmolyzed cytoplasm contains dark-staining granules. × 1130

C.—Four-day infection. Group of invaded cells. The cell *a* is shrinking. The misshapen red-stained plastids are conspicuous, but the cytoplasm is not granular. The invaded cells at *b* and *c* show slight disturbance. × 730

D.—Five-day infection. Longitudinal section through margin of mycelium and tissues beyond. A few invaded cells dead (at *a*). The host cells within the infection that are alive are turgid, and show normal staining reactions. Beyond the mycelium (from *b* to *d*) the cells are markedly plasmolyzed, many of the nuclei are dead, and the plastids are flattened into disks which seem to be arranged in chains × 333

the host cells. The results of the first attacks of the three forms on Khapli emmer are somewhat similar. This first intimate relationship between the fungus and its host results in the sudden and violent collapse of the host cells involved. In an infection three days old there may be three or four dead host cells. The later reactions of the host vary somewhat according to the form used.

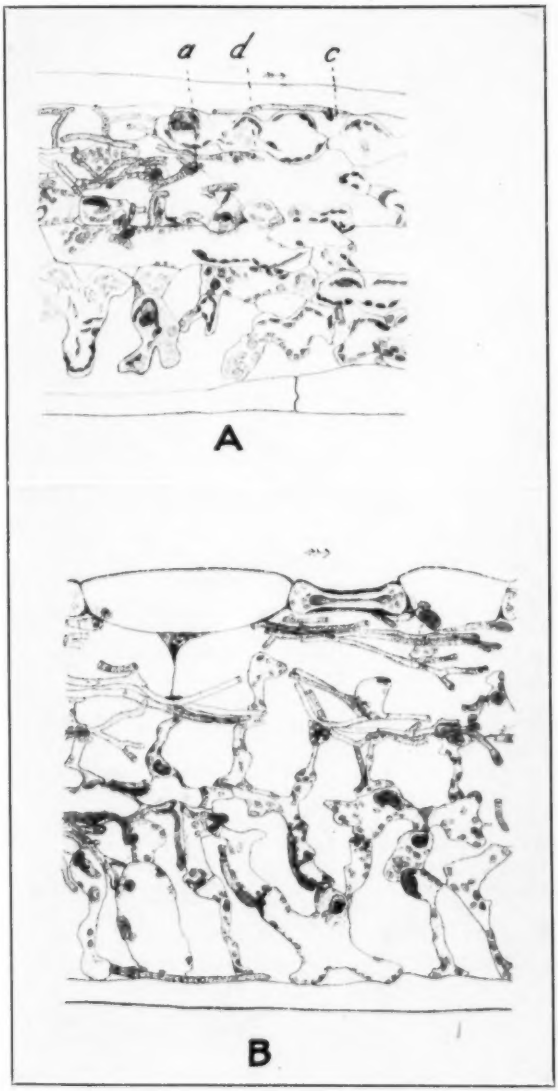
Reaction to Form 9

Something of the appearance of the collapse from form 9 is shown in Plate 9, A and B. These areas, however, are taken from five-day material. Plate 9, A, represents a partially collapsed cell, within which the haustorium *b* is still visible. The cell wall has retained nearly its original shape, but the cell contents are greatly plasmolyzed. The central vacuole of the cell has been almost obliterated as the protoplast contracted. The nucleus *a* also has contracted. The plastids are still discernible and are but little reduced in size, but both in their shape and staining reaction there is evidence of dissolution. Their strong affinity for saffranin at this stage is perhaps indicative of a breakdown of their substance into organic acids. In Plate 9, B, the collapse is complete and the cell contents have changed into a nearly homogenous red-staining mass. With the triple stain it is not easy to distinguish the cell wall, but by staining with saffranin and light green, the wall can be seen as a fine green line outlining the red contents. The wall is neither swollen nor dissolved.

The haustoria in these collapsed host cells do not expand normally, and the fungous plasm of which they are composed dies with the death of the cell in which they are found. In spite of these apparent failures of the fungus in its earlier attempts, however, it gains slowly in strength, thus indicating that it must have obtained a little food through these short-lived haustoria. No cases have been seen in which the fungus dies at this stage.

In Plate 9, C, is a bit of the advance growth of a 3-day mycelium. It has left behind two dead hosts cells (not included in the drawing), yet the hypha is vigorous and apparently well fed. At *a* it formed a haustorium mother cell which gave rise to the haustorium *b*. The latter has not expanded normally, the host cell is dying, and the haustorium mother cell at *a* has collapsed and now stains deep red. These shrunken, wrinkled red-stained haustorium mother cells of dead haustoria are abundant in young mycelia. In spite of this reverse, the hypha grew on and formed another haustorium mother cell of perfectly normal appearance, at *c*.

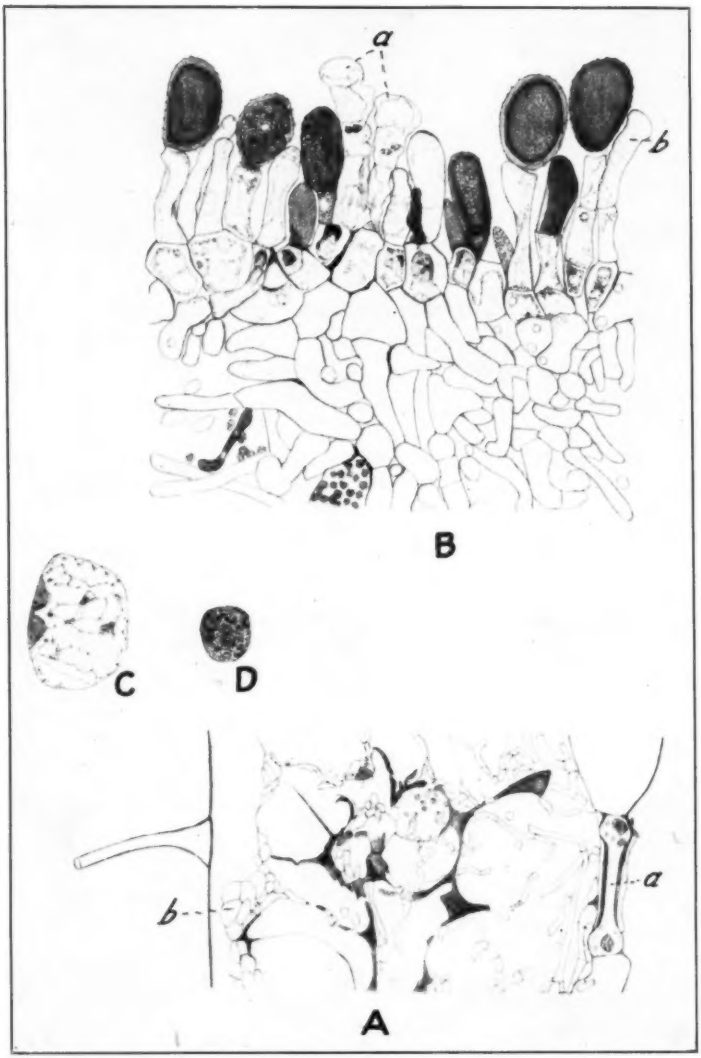
A little later, the reaction of the host to the fungus is less violent. An occasional host cell collapses quickly when invaded on the fourth or fifth day (pl. 9, A and B), but many of the host cells containing haustoria remain turgid and nearly normal in appearance. Due either to a change in the fungus or in the host, the substances that pass out of the haustorium into the host cell no longer effect its rapid destruction. In fact, the host cell may live on for several days or even a week. In Plate 2, E (5-day), at the lower end of the drawing, many of the invaded cells contain well-developed haustoria, but are still thriving in appearance. At this time the fungus has made a fairly good growth. Sooner or later, however, the majority of these cells collapse in much the same fashion as did the first one.



Form 21 of *Puccinia graminis tritici* on Khapli emmer

A.—Six-day infection. Fungus growing out through the zone of dying tissue. Stolons at *a*, *d*, and *c*. × 333

B.—Nine-day infection. The stolons of the fungus have traversed the dying tissues and reached fresh green tissues on beyond. × 333



Form 21 of *Puccinia graminis tritici* on Khapli emmer

A.—The center of 10-day infection. Fungus entered at *a*. Host cells shrunken to thin, irregular, red-stained masses. Hyphae massed below epidermis in places (as at *b*), but no spore formation. × 333

B.—Fourteen-day infection. Portion of uredinium, with under-sized spores that are well formed and others (at *a* and *b*) empty and shriveled. × 730

C.—Large host nucleus (18 × 13.5 μ) in an epidermal cell adjoining mycelium in a 10-day infection. × 1130

D.—Normal epidermal nucleus from same slide as C. Small and dense. × 1130

Reaction to Form 21

This physiologic form of the fungus causes a quicker and more violent death in the host tissues than either of the other two. The first haustorium in a mesophyll cell produces immediate evidence of disturbance. The haustorium remains small, the host nucleus moves over to it, the plastids become altered chemically in some fashion (as evidenced by strong affinity for saffranin), and the whole cell collapses in much the same fashion as was described for form 9.

By some adjustment of host or fungus, or both, the later haustoria in mesophyll tissue cause a slower disturbance in the cells invaded. To be sure, these cells collapse and die ultimately, and their final appearance is similar to that of the first cell killed, but the second and third cells invaded by the fungus react less rapidly and violently than the first, and the later ones still more slowly. As the interval lengthens between invasion and collapse the haustorium attains fuller development, extracts more food from the host, and the fungus gains in strength. In an infection three days old, there are from one to six dead mesophyll cells, but there may be one which is still normal in appearance although it contains a half-grown haustorium. In spite of the difficulties in its becoming established, no case has been noted in which the fungus dies at this stage.

During the next (or fourth) day the mycelium gains more rapidly. The older hyphae near the point of entry are drained and empty, but the freshly growing tips look vigorous. An occasional haustorium mother cell is flattened and wrinkled and red-stained, but the majority are clear and plump. The host cells in which the fungus has formed haustoria are in all stages of collapse. The number of fully collapsed cells varies from a few to more than a dozen; other cells are partly collapsed; still others have contents only slightly disturbed; and a few appear uninjured, although containing good-sized haustoria.

The dying mesophyll cells vary in appearance. In this material the degenerating protoplasm often is coarsely granular. In Plate 4, B, the fungus entered the cell at *a*, producing the haustorium *b*. The haustorium remained small and dense, and the mother cell *a* outside has collapsed. The nucleus of the host cell *c*, as has been noted before in cases where rust and host are uncongenial, has moved over to the haustorium and flattened against it. The cytoplasm has undergone degenerative changes and has contracted into an irregular red-staining mass dotted with darker staining granules. A few plastids are still discernible.

In Plate 4, C, the dying cell *a* is a quite different type. The swollen, deformed, red-staining plastids form the conspicuous part of the shrinking protoplast. There are few if any granules. At *b* and *c* are newly invaded cells containing small haustoria. These cells show only slight disturbances. In each case, the nucleus and a part of the cytoplasm have moved over to the haustorium, but the cells are still living. The final state of the invaded cells which collapse (pl. 4, D, at *a*) is indistinguishable from that of form 9.

Reaction to Form 27

Only the first cells attacked by the fungus collapse promptly. No case has been noted in which the fungus dies as a result of these losses.

After the death of the first two or three host cells, the reaction to the fungus becomes more gradual. In Plate 7, A, taken from a 5-day preparation, the haustorium mother cell *a* is empty, showing that its entire contents have moved in to form the haustorium *b*. The latter has not expanded, however, but still is small and dense. The host cell is dying, but there has been no sudden sharp collapse of either cell contents or wall. The contents are partly plasmolyzed, and the dead nucleus *c* is homogenous. In some cases the contents are granular in appearance. This slow collapse and solution of the cell contents is the main type of reaction at this stage. There may be as many as a dozen cells in this condition in a five-day infection, and several more the following day.

There is also at this time evidence of the beginning of a still slower reaction of the host to the fungus. Plate 7, A, shows an example of this type. The haustorium *d* has expanded vigorously, and its cytoplasm has the open reticulated structure of a healthy organ. The host cell, too, has normal appearance and arrangement of cell contents, except that the nucleus has moved over to the haustorium.

The number of cells that succumb during the early history of the fungus is lower here than with either of the other rusts. In a six-day infection about a dozen or more cells have collapsed and a dozen more show injury.

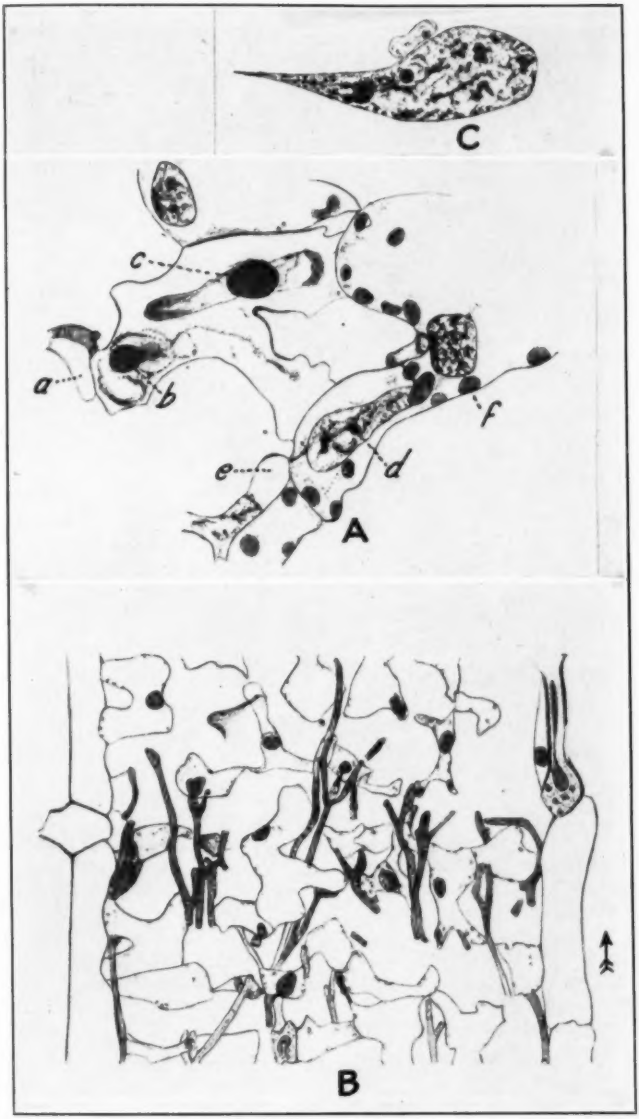
With all three forms of the fungus on Khapli emmer more or less adjustment to the disease takes place, and the host gives the appearance of being more susceptible to the fungus after a few days than at the outset. This adjustment, the chemical nature of which is only a matter of conjecture, is most rapid with form 27, intermediate in form 9, and slowest in form 21.

COMPARISON OF DETAILS OF VEGETATIVE GROWTH OF THE THREE FORMS

These three forms of stem rust are very similar morphologically. They may differ somewhat in vegetative vigor, when on Khapli emmer at least, although this difference is less than would be supposed from the final size of the flecks they produce.

HYPHAE

Vegetative hyphae have about the same diameter in all three races. Fresh growing hyphae in young mycelia average 2.1 μ in diameter (Table IV) and in older mycelia the growing tips average 2.4 μ in diameter. In all three forms the active vegetative life of any one portion of a hypha is short. It soon becomes drained of all stainable contents. In the process of drainage, the fungous cytoplasm disappears first and later on the nuclei. In the nucleus, the nucleoli are digested before the chromatin. Finally the hypha is clear and remains as an empty tube, the wall of which stains strongly with orange. As the living substance of an older part of a pypha passes out towards the growing tip, the empty tube increases in diameter. Drained hyphae of all three forms range from 2 or 3 μ up to 6 or 8 μ in diameter, and in the neighborhood of a uredinium they may be even larger (pl. 3, C, and pl. 6, B). This is not accompanied by an increase in thickness in the wall itself.

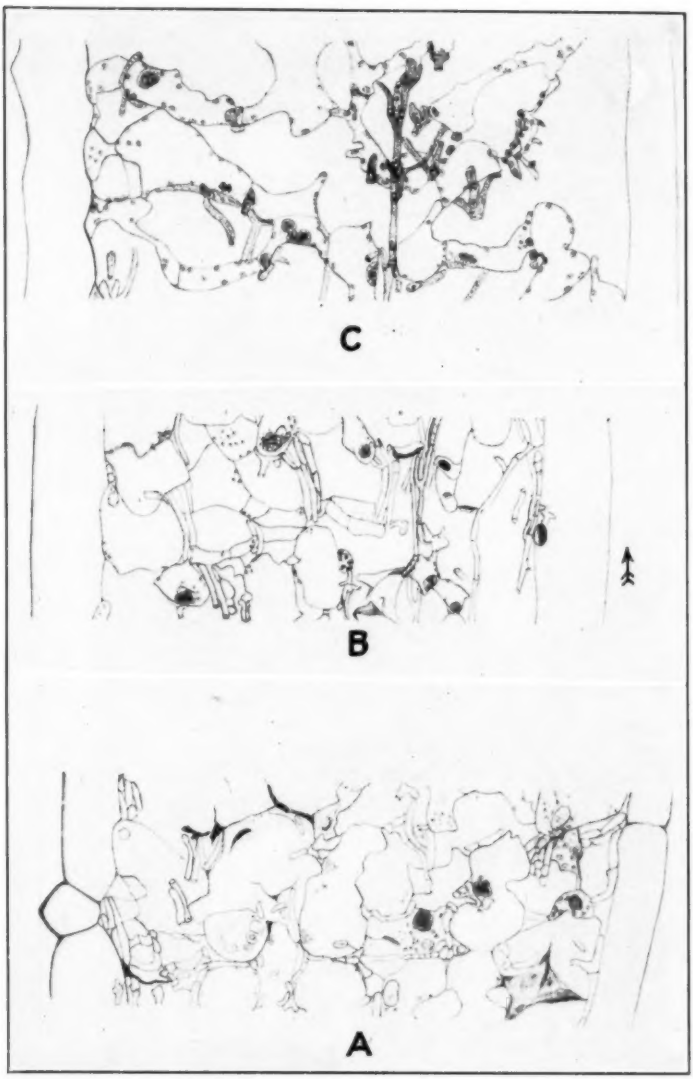


Form 27 of *Puccinia graminis tritici* on Khapli emmer

A.—Five-day infection. Attacked cells. Haustorium mother cell *a* formed haustorium *b*. Latter remained dense, and is dead. Host cell is dead, with nucleus shrunken and homogeneous (*c*). At *e* another mother cell formed haustorium *d*, which expanded fully. Host cell vigorous, and its nucleus (*f*) has moved to the haustorium. × 1130

B.—Nine-day infection. Fresh marginal growth of the fungus pushing out through the impoverished region. Host tissue living. × 333

C.—Giant host nucleus (40.5 × 11.3 μ) in an epidermal cell adjoining a 12-day infection. × 1130



Form 27 of *Puccinia graminis tritici* on Khapli emmer

A.—Eighteen-day infection. Narrow strip through longitudinal section of leaf, from surface to surface, near center of infection. Mycelium drained. No trace of spore formation. Rather meager growth. A few host cells sharply collapsed (dark streaks), the rest retaining shape, but empty or nearly so. × 333

B.—Same infection, about halfway out to edge of mycelium. Empty stolons of the fungus run longitudinally. Host cells nearly empty, but sometimes living. Not collapsed. × 333

C.—Same infection. Marginal growth of hyphae. Host tissues living, but plastids reduced in size. × 333

TABLE IV. *Comparison of hyphae, haustoria, and haustorium mother cells of forms 9, 21, and 27 of wheat stem rust, on Khapli emmer*

	Age of fungus	Form 9	Age of fungus	Form 21	Age of fungus	Form 27
	Days		*Days*		*Days*	
Diameter of hyphae; each measurement represents the average of 10.	4	2.1 μ hyphae with protoplasm.	4	2.2 μ hyphae with protoplasm.	5	2.1 μ hyphae with protoplasm.
	8	2.4 μ hyphae with protoplasm. 2.9 μ hyphae drained.	8	2.1 μ hyphae with protolpasm. 3.7 μ hyphae drained.	8	2.4 μ hyphae with protoplasm. 2.8 μ hyphae drained.
	12	2.4 μ hypahe with protoplasm. 3.9 μ hyphae drained.	14	2 to 6 μ hyphae drained 8.0 μ near uredinium.	12	2.2 μ hyphae with protoplasm. 3.0 μ hyphae drained.
			18	3.8 μ partly drained. 5.8 μ largest drained hyphae; not near uredinium.	18	2.3 μ hyphae with protoplasm. 4.3 μ hyphae drained.
Average size of 10 largest haustoria found.	5 6	13.2 by 4.8 μ.	5 6	13.7 by 4.6 μ.	5	17.1 by 5.0 μ.
	8	14.6 by 5.6 μ.			10	14.4 by 5.0 μ.
		Up to 20 μ in epidermal tissue.	------	In epidermal tissue up to 26.0 μ.		The larger epidermal haustoria averaged 16.4 by 6.0 μ; largest noted was 19.5 by 5.2 μ.
Haustorium mother cells.		Majority of haustorium mother cells affected. In younger mycelia they are wrinkled and collapsed and stain with saffranin. In older mycelia the mother cell wall is greatly swollen and takes no color in triple stain.		Not more than 5 per cent of the mother cells with swollen walls in young mycelia. A larger number in 14 and 18 day material.		Practically no swollen walls. Occasionally a haustorium mother cell is wrinkled and red-stained. None noted with swollen walls, save in one plant of 14-day material. (Possibly host was rogue.)

HAUSTORIA

In general, the haustoria are undersized. As already stated, the first-formed haustoria in mesophyll tissue are invariably small and dense and soon die. Later haustoria of all three physiologic forms expand, and their cytoplasm opens out into a reticulum, but they never attain full size. The average of the 10 largest found in form 9 (Table IV) was 14.6 by 5.6 μ; in form 21, 13.7 by 4.6 μ; and in form 27, 17.1 by 5.0 μ.

In bundle sheath cells the haustoria are larger than in mesophyll cells; and in epidermal tissue haustoria attain full size, even in young infections. Plate 4, A, shows a vigorous haustorium. It was found in an epidermal cell in a four-day mycelium of form 21, and is 26 μ long. In Plate 4, B, taken from the same slide and drawn to the same magnification, is the small dense haustorium *b*, in mesophyll tissue, showing the contrast between the two at that age.

HAUSTORIUM MOTHER CELLS

Evidence of physiologic differences in the three fungi is to be found in the haustorium mother cells (Table IV).

In form 9, the majority of the haustorium mother cells undergo modification. A normal vigorous haustorium mother cell of a 3-day mycelium just ready to form a haustorium is shown in Plate 9, C, at *c*. It is plump and thin-walled, and contains rich cytoplasm and two minute nuclei. Plate 9, C, at *a*, shows the appearance of the mother cell just after its protoplasm has passed into the host cell. The empty mother cell has lost its normal turgor. It is wrinkled and collapsed. Its wall is altered chemically, for it now stains with saffranin instead of orange.

A small portion of a mycelium (fixed four days after inoculation) is represented in Plate 9, D. The mother cell *a* formed the haustorium *b*, which has expanded partly and appears to be living. The invaded host cell is still living and turgid. The haustorium mother cell *a* has flattened out, but is clear and no longer stains with either saffranin or orange. In slightly older material (pl. 9, E) the wall of the haustorium mother cell swells up, takes on a glassy appearance, and loses its affinity for the usual stains. With ruthenium red—a pectose stain—the wall stains pink. Plate 9, F, G, and H, and Plate 2 A, B, and C, show varying aspects of this reaction, as seen in still older (10-day) infections. In Plate 9, F, the cell *a* is still normal, and the haustorium *b* appears to be functioning. In Plate 9, G, is shown the most common modification. The bulk of the cell seems to be occupied by swollen wall material, leaving only a narrow slit running through the center of the cell. In Plate 9, H, and Plate 2, A, progress of the reaction is more uneven, leaving irregular clear spaces. In Plate 2, B, the cell lumen is quite obliterated, the only remaining trace of it being a line down the center which refracts light differently from the rest. The average size of these swollen cells is 10.0 by 4.5 μ. In Plate 2, C, is an unusual and unexplained case in which the haustorium itself is glassy in appearance and looks much the same as the altered mother cell.

In exceptional cases in form 9 the hypha just below the haustorium mother cell is similarly affected. In Plate 2, D, taken from a 12-day infection, the hypha first produced a haustorium mother cell *a*, which gave rise to the haustorium *b*, then pushed out branches, *c* and *d*, the latter of which produced a second mother cell *e*. The older mother cell *a* and the haustorium produced by it, *b*, are nearly dissolved, and are to be distinguished only by faint outlines. In the hypha below and its lateral branches the walls are swollen, colorless, and glassy in appearance, in fact, closely similar to modified haustorium mother cells. The younger mother cell *e* is collapsed and red-stained.

In form 21, on the same host, the reaction is much less marked. In the first period of the growth of the fungus while the attacked host cells are dying rapidly, an occasional haustorium mother cell collapses (pl. 4, B at *a*). Usually, however, they remain plump and turgid (pl. 4, A at *a*, and C at *c*). During the main period of development of the fungus not more than 5 per cent of the mother cells shows the swollen glassy-looking wall common in form 9. This reaction usually increases with age, and in old material (14 or 18 days) a somewhat larger number are found.

EXPLANATORY LEGEND FOR PLATE 9

A.—Five-day infection. Invaded cell. Small, dense haustorium at *b*. Protoplast plasmolyzed, and wall beginning to collapse. Plastids dying; saffranin positive. × 1130

B.—Five-day infection. Dead cell. Completely collapsed. Adjoining cells are not shrunken, but the nucleus of one (*a*) has moved to surface of contact. × 1130

C.—Advancing tip of 3-day mycelium. Vigorous haustorium mother cell at *c*. Older mother cell *a* formed haustorium *b*. Host cell and haustorium dying, and mother cell shrunken and red-stained. × 1130

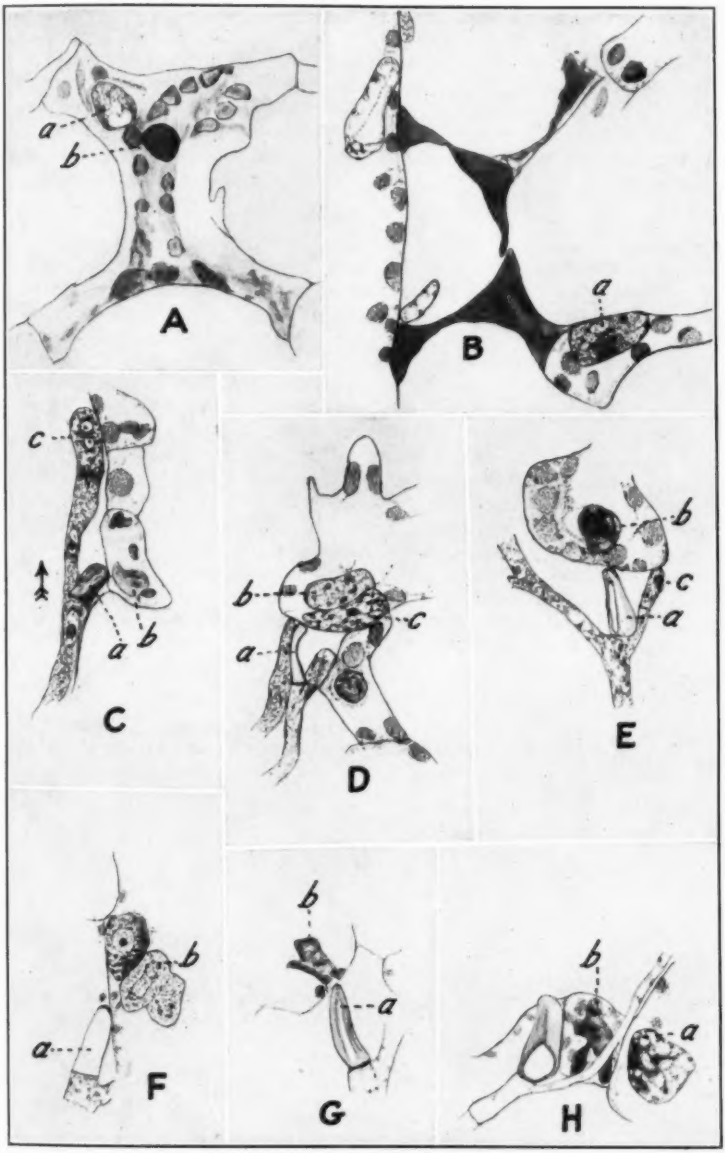
D.—Four-day infection. Haustorium mother cell *a* produced haustorium *b*. Host nucleus *c* has pushed in between and broken neck of haustorium. The mother cell is flattened, but clear. × 1130

E.—Five-day infection. Haustorium mother cell *a* with wall greatly swollen, reducing the lumen to a narrow slit through the center. × 1130

F.—Ten-day infection. Normal mother cell at *a*, expanding haustorium *b*, and host nucleus pushing between them. × 1130

G.—Ten-day infection. Mother cell *a* with a swollen wall. Dead haustorium (*b*). × 1130

H.—Ten-day infection. Mother-cell wall irregularly broken, obliterating distal half of lumen. Haustorium *b* collapsed and misshapen. Nucleus *a* of adjoining cell has moved to contact wall. × 1130



Form 9 of *Puccinia graminis tritici* on Khapli emmer

For explanatory legend see page 714

In form 27, hardly a trace of this reaction is to be found. Rarely a mother-cell wall stains red, indicating the initial stage of breakdown. In Plate 7, A, are two mother cells, *a* and *e*, one in connection with a dying cell, the other with a living cell. Both mother cells are thin-walled and turgid, and have the appearance that they would have in a congenial environment. In one seedling of 14-day material a number of mother cells with swollen walls were found. This remains unexplained, for in still older material (18 days) no trace of this reaction was noted.

COMPARISON OF HOST NUCLEI

It has been noted previously that host nuclei in infected areas may become altered in size. In the cases studied (*2*, *3*), if host and rust are congenial, the nuclei undergo a marked expansion. Data obtained thus far indicate that when the host is completely immune, its nuclei expand but slightly.

Khapli emmer shows an intermediate type of infection, being resistant to, but not fully immune from, stem rust. The fungi do not thrive, but live on and spread slowly and perhaps produce a few spores. The reaction of the host nucleus to this type of infection has been studied. Measurements of mesophyll nuclei in infections on Khapli (Table V) show that from all three forms there is expansion of the nuclei in young (five-day) infections. It is not uniform, some nuclei being considerably enlarged; in fact, paralleling those in susceptible hosts, while others remain unchanged. The average is about the same from all three forms, and, on the whole, is somewhat less than in susceptible hosts. The amount of expansion does not increase with age of the infection. On the contrary, from form 27 at least (Table V), there is slightly less expansion of the nuclei in older infections than at five days, and rarely does one find nuclei of normal size and appearance in cells containing old haustoria.

TABLE V.—*A comparison of host nuclei in infections by physiologic forms 9, 21, and 27 of stem rust of wheat, on Khapli emmer*

	Age of infection	Form 9	Age of infection	Form 21	Age of infection	Form 27
	Days		*Days*		*Days*	
Mesophyll nuclei: Each figure is the average of 10 nuclei.	5	8.2×6.5 μ normal nucleus.	5	8.4×6.1 μ normal.	5	8.8×6.6 μ normal.
	5	10.2×7.4 μ in infection.	5	10.0×6.9 μ in infection.	5	10.0×8.0 μ in infection.
	7	9.9×7.2 μ in infection.	7	9.7×7.5 μ in infection.	7	10.0×8.1 μ in infection.
	8	10.0×7.5 μ in infection.	9	9.2×7.4 μ in infection.	10	9.8×7.5 μ in infection.
	10	9.2×6.3 μ in infection.	10	9.5×7.0 μ in infection.	12	9.3×7.5 μ in infection.
	12	10.4×6.8 μ in infection.				
Epidermal nuclei: Normal		8.7×6.6 μ				
Average of 10 largest found in 8, 10, and 12 day infections.		17.4×7.6 μ		16.2×9.3 μ		17.4×8.7 μ

Nuclei of epidermal tissues react very differently from this. The ordinary epidermal nucleus in healthy tissue averages 8.7 by 6.6 μ (Table V). In epidermal tissue adjoining infections, an occasional nucleus undergoes enormous expansion. In Plate 6, C, is drawn a

giant nucleus in an infection by form 21, and in Plate 6, D, at the same magnification, a normal epidermal nucleus. In Plate 7, C, is drawn the largest epidermal nucleus seen. It was taken from a 12-day infection by form 27 and measures 40.5 by 11.3 μ. This expansion is more than an intake of water, for there is a marked increase in the amount of chromatin present. The shape of these giant nuclei varies. Some are ellipsoidal, while others are drawn out at one or both ends to long tapering points. By no means all of the epidermal nuclei within range of the fungus undergo this change, for some of them keep their original size and shape.

COMPARISON OF THE HOST TISSUES JUST BEYOND THE MYCELIAL AREAS

A study of the host tissues of Khapli emmer just beyond the mycelial areas brings out several differences. Secondary damage occurs from all three forms, but the appearance and extent of the damage differ.

Reaction to Form 9

In infections by form 9 on Khapli emmer, the zone of tissue surrounding an infection shows no plasmolysis. Even the cells in contact with a cell killed by the fungus remain turgid (pl. 9, B). These cells are not visibly injured, but there may be some slight disturbance in them, for the nucleus (pl. 9, B at *a*, and H at *a*) often moves up to the surface of contact. Plate 2, E, represents a portion of a longitudinal section of the leaf through part of a five-day mycelium and the tissues beyond it. The outer tissues look vigorous. Only by exact measurements can the effect of the fungus beyond the outermost hypha be detected. The average plastid in living cells containing haustoria measures 2.9 by 2.1 μ, the plastids at a distance of two cells beyond the edge of the mycelium average 3.2 by 2.0 μ, and the normal healthy plastids about 3.3 by 2.5 μ.

The further progress in the reaction in the outer zone in this case consists of a rapid decrease in the cell contents for a considerable distance beyond the fungus. Table III records changes in plastid size. Perhaps some substance which is either secreted by the fungus or formed secondarily in invaded host cells as a result of the secretions of the fungus is diffusing rapidly into tissues remote from the fungus itself.

In Plate 3, A, is drawn a narrow strip from the upper to the lower surface of a leaf through the center of an eight-day infection. Plate 3, B, shows a similar strip through the dead area that surrounds this infection. This fungus entered the stoma at *a*, Plate 3, A, and killed the first cell attacked, *b*, and several others which lay in adjoining sections. The cells invaded later have survived and are still green and active.

Beyond the limited primary region occupied by the fungus the host tissues are dying rapidly. The manner of death, however, is very different from the quick collapse seen in the infection itself. Here (pl. 3, A at *c*) there is no collapse and no change in the staining reaction of the plastids. The nucleus and cytoplasm retain their normal position against the cell wall, and the plastids continue to stain orange or faintly bluish with the triple stain, as they become smaller and fainter, and disappear. The nucleus also shrinks and disappears. The cell wall may retain its original shape for a short time after the contents have been dissolved, but eventually it, too, is partly digested. This digestion of the host cell walls takes place

without swelling. No case has been seen of swollen walls. Farther away from the fungus (pl. 3, B) the cells are dead, the cell contents are digested, and even the walls are nearly gone, only a few faint shreds remaining. This dead area takes little or no stain, and it contrasts sharply with the intensely stained, homogeneous contents of the dead, collapsed cells of the central region.

Not all cases are as extreme as this. The digestion may stop at an earlier stage, leaving the cells enfeebled but living. They may even revive slightly under the direct stimulus of the fungus as it grows through this area.

Reaction to Form 21

In contrast with form 9, form 21 produces marked plasmolysis in the outer tissues. The first hint of this was seen in one or two infections on the third day, when cells beyond the fungus itself, but lying in contact with invaded cells, showed the initial stages of plasmolysis. Usually this does not occur until the fourth day, and sometimes not until the fifth. Once this change has begun, however, it usually spreads rapidly, and soon a broad zone surrounding the fungus has a characteristically altered appearance.

Plate 4, D, represents a section through the margin of a five-day mycelium and the area beyond it. It is of the same age as the form 9 shown in Plate 2, E, and was grown under parallel conditions, but contrasts sharply with it. At *a*, not far from the central part of the infection, are collapsed dead cells. Out in the area occupied by the younger growing hyphae from *a* to *b* the host cells are turgid; the plastids, though somewhat reduced in size, have their usual size and position, and they stain a faint orange or a bluish tint, like the plastids of healthy tissue. Beyond the fungus, in a broad zone from *b* to *d*, the cells are plasmolyzed, the nuclei are often dead and disorganized, and the plastids are flattened and stain with saffranin and are often arranged in chains. These plastids are about the same size as before, but are flattened into disks. The dimensions are 4.3 by 1.4 μ (Table III). These changes are not accompanied by any massing of cytoplasm on the side of the cell, either toward or away from the fungus. The intense red stain of plastids and nucleus in this zone makes the cytoplasm conspicuous, even with the low power of the microscope.

The extent of the saffranin-stained zone is variable. In a few cases there is none at all. In a few more only an occasional cell is so affected. In one five-day infection, on the other hand, the diameter of the mycelial area is 300 μ, and the diameter of the mycelial area plus the plasmolyzed area is 700 μ.

These plasmolyzed tissues usually die promptly. Even in five-day preparations some of the nuclei in this zone are dead and of course cells with dead nuclei can not live. The question as to whether any of these cells can recover is not easily decided. Since the width of this outer zone varies greatly in different infections, and often extends out irregularly, involving some cells and not others, and since any given preparation can be studied at only one stage in its development, it is not easy to determine whether a living cell in this area was never injured or had shrunk and later recovered. In general, however, the plasmolyzed cells soon collapse and die and show in stained preparations as thin irregularly branched red-stained

masses indistinguishable in appearance from the dead cells at the center of the same infection (pl. 6, A).

This rapid formation of a limited zone of damaged tissue beyond the confines of the hyphae took place during the first few days of the growth of the fungus. Nothing corresponding to this occurs in the later history of the fungus when once more it is growing in fresh tissues beyond this zone. The hyphae (as in pl. 5, B) seem fairly vigorous and form haustoria, but no new plasmolyzed saffranin-stained zone of cells has been noted beyond the later limits of the fungus.

Within the range of the fungus itself the tissues soon die. The cells in which haustoria form collapse rather promptly, so as the fungus spreads radially at the periphery the diameter of the mycelium is not much greater than that of the dead area it has produced.

Reactions to Form 27

In the six-day infection, no visible changes in the tissues beyond the fungus have been detected. On the seventh day distinct impoverishment is noted throughout an area half a millimeter wide. Usually no plasmolysis occurs here, and no change in the staining reaction. An occasional hint of red-staining plasmolyzed cells has been noted at the edge of the mycelium but hardly enough to warrant a positive statement.

This outer zone sometimes extends a little farther in the next few days, but often remains about the same. Its cells usually are living, though at a low ebb of activity.

An interesting point concerning this impoverished region is occasional evidence of variations in the host cells. It is not uncommon to find little patches of cells here and there far beyond the fungus which are in considerably better condition than the rest. Plastids in such cells averaged 3 by 1.9 μ, as contrasted with 2.5 by 1.3 μ in the cells on all sides of the patch. It is not clear just how and why these cells are able to maintain themselves when tissues on all sides of them and at greater distances from the fungus are nearly killed. It may happen, too, that this altered outer zone of tissue extends farther near one surface of the leaf than at the other. In other words, the fleck often is larger as seen from one surface of the leaf than it is on the other.

The quick collapse of a cell into a stringy, red-staining body, so common around infections by form 21, is rare here. Even in old infections (Table III) not more than 10 per cent of the cells die in that fashion. A few cells are empty and clear. The great majority are still living and revive under the direct stimulation of the fungus as it passes through. The plastids are larger here at 14 days than at 8 days.

The secondary, pale area found in old infections resembles the primary. Here, too, there is little or no collapse of the cells but merely impoverishment. In general, with form 27 the damage beyond the fungus is more widespread, but milder in character, than with the other two forms.

COMPARISON OF REPRODUCTIVE ACTIVITIES

The three physiologic forms, when grown on the same host under parallel conditions, show differences in reproductive activity. None of them produces normal uredinia. The sori are minute at best.

In Form 9

In many infections there is no attempt at spore formation. In some cases, however, the fungus grows more vigorously and produces a minute sorus. The first hint of this is seen on the seventh day, when, in a few cases, the hyphae begin massing below the epidermis. The uredinium may break the epidermis and shed a few spores, or it may remain submerged. A uredinium that has broken the surface at one point may extend into a pocket in the interior of the leaf. Plate 3, C, represents a section through such a pocket taken from an 18-day infection. Both host and parasite are dead. The whole surface of the little cavity is lined with spore-bearing tissues, and the ill-formed spores extend into and nearly fill it. Perhaps the loss of turgor in the host tissues reduces the leverage of the fungus and renders ineffective its efforts to push up and break through the epidermis.

In Form 21

Relatively few spores are produced by form 21 on Khapli emmer. Sometimes there is no trace of spore formation. Sometimes—about the seventh day—hyphae mass under the epidermis as though in preparation for spore formation. It is not unusual, however, to find in slightly older preparations evidence that the reproductive process has been begun and then abandoned before any spores have been formed. In Plate 6, A, at *b*, at the center of the infection, are several layers of large hyphae, now dead and empty, which represent an abandoned attempt at reproduction.

Sometimes, on the contrary, the process is carried through, and a minute uredinium is formed. The process is not fully successful, for many of the spores are abortive (pl. 6, B, at *a* and *b*) and the well-formed spores of this uredinium are undersized (about 20 by 12 μ).

In Form 27

Form 27, on Khapli, exhibits the greatest vegetative activity and the least reproductive activity of the three forms. In fact, a careful study of older infections failed to reveal more than a trace of even the initial stages of uredinium formation. Plate 8, A, is typical of the appearance at the center of old infections. The fungus growth is sparse, not filling the intercellular spaces, and there is only a suggestion of massing of the hyphae at the epidermis.

DISCUSSION

Microscopic study of several races of wheat stem rust on a resistant host brings to light physiological differences in the rusts. In forms 9, 21, and 27, on Khapli emmer, some of these are differences of degree rather than of kind.

The dissimilarities noted probably do not represent the entire range of differences in these physiologic forms. It is quite possible that differences which do not find expression here (in ways visible under the microscope, at least) might be brought out clearly on another host. It is conceivable that substances secreted by the fungus might be rendered inactive by one host and not by another, or even that the secretions themselves might vary according to the

food present in the host on which it is grown. But even in this limited study there is enough evidence of difference to show that these physiologic forms of the rust are well individuated.

For greater convenience in estimating and balancing similarities and differences, the principal data about these forms have been summarized in parallel columns in Table VI. One of the first points which stands out clearly on comparison of the three is that not all of the extreme reactions occur in the same rust. Each of the three is mild in some respects, intermediate in others, and extreme in still others.

The appressoria of all three forms of the fungus secrete some substance, which, if present in sufficient amount, penetrates and kills the guard cells and alters their walls. So far as can be judged by appearances, it is the same substance in all three, but varies in quantity from a mere trace in form 9 to a considerable amount in form 27.

The utility of this secretion is not obvious. On comparing the percentage of entries with the degree of stomatal injury, it is to be noted that where injuries are least serious the entries are greatest in number. It is doubtful how much weight should be given to this, but at least there is little ground here for the idea that the substance secreted by the appressorium assists the entrance of the fungus through the stoma. Possibly, however, the appressorial secretion is a survival from an earlier period in the evolution of the fungus when it did secrete enough to dissolve its way into the host.

No one of these forms, in its later development, produces just this effect upon cell walls and cell contents in the interior of the leaf of Khapli emmer. Either this substance is secreted only during the time when the fungous plasm is in the appressorium, or, if secreted later, when the chemical composition of mesophyll walls and protoplasm is such that they are not altered in the same way by it. Form 27, which does the greatest damage of the three to the guard cells at the time of entry, does the least violence to tissues during early development within the host. This suggests that the later secretions of this fungus may be different in character and amount from that of the appressorium.

As pointed out by Loftfield (*14*) and discussed by Hursh (*9*) and the present writer (*3*), the tendency in cereals is to operate with many closed stomata, and on some days the stomata do not open. This may be an important factor, but probably not the only one, in determining the percentage of entries.

In all three forms of the rust, the first mesophyll cells attacked by the fungus are killed outright, and in all three a gradual change takes place by which invaded cells survive longer and show to some extent the stimulated condition present in infections on susceptible hosts. The cells maintain turgor for a while, their nuclei become enlarged, and the reduction in plastid size is checked. The time required for this adjustment and the degree to which it takes place differ in the three forms. At first sight, at least, this change looks like a limited increase in susceptibility.

It is not known how common this reaction is. Mindum wheat infected by form 3 of stemrust (*3*) shows a similar but less pronounced change of the same sort, and still other cases studied (unpublished data) are not unlike it.

TABLE VI.—*Summary of data on three forms of stem rust of wheat, 9, 21, 27, on Khapli emmer*

Data on—	Form 9	Form 21	Form 27
Entries	52 per cent of the fungi enter the host.	42 per cent of the fungi enter.	21 per cent of the fungi enter.
Damage to stomata through which the fungus enters.	Very slight damage	Medium	Extreme.
First attacks	The first mesophyll cells invaded by the fungus collapse and die quickly. The fungus survives.	Similar to 9	Similar to 9.
The adjustment between fungus and host by which their relations seem to become more congenial.	Medium time for adjustment. Later cells invaded by the fungus collapse more and more slowly until finally a cell can remain turgid and the haustorium within it expand partly and function several days before the host cell collapses.	Slow adjustment. More toxic than the other races. There is more dead tissue in infections of a given age than in either of the others.	Rapid adjustment. Very few host cells killed. Relation between host and fungus soon appears fairly congenial. Very few host cells collapse even in old infections.
Haustoria	In epidermal tissue haustoria become full sized. In mesophyll tissue the first haustoria are small and dense. Later ones expand partly.	Similar to 9	Similar to 9.
Hyphae	Older drained hyphae undergo marked increase in diameter.	Same	Same.
Modification of haustorium mother cells.	Extreme. The majority of haustorium mother cells in young fungi become wrinkled and flattened and in older fungi they become greatly swollen.	Slight. About 5 per cent of the mother cell walls become swollen. More in very old infections.	Virtually absent. Practically no haustorium mother cells with swollen walls.
Host nuclei	Nuclei undergo moderate expansion in infected mesophyll tissue. Occasional epidermal nucleus is enormously expanded.	Same	Same.
First discoloration of tissues visible in living infections.	Fourth or fifth day after inoculation.	Fourth day	Fifth or sixth day.
First appearance (seen under the microscope) of damage to host tissues beyond the fungus.	Fifth or sixth day after inoculation.	Third to fifth day	Sixth or seventh day.
Character of damage to host tissues beyond the fungus.	Impoverishment. No plasmolysis or collapse or alteration in staining reaction. Host cells become poorer and poorer in content until they are nearly or quite empty and take no stain.	Collapse. The contents of a cell become plasmolyzed and collapse into a little irregular body that stains intensely with saffranin.	Impoverishment. Similar to 9 but less extreme. Cells usually live.
Later history of outer zone.	Fungus grows out through the damaged zone. Enfeebled cells sometimes recover slightly under the stimulus of direct contact with the fungus.	Fungus grows out through damaged zone. Host tissues do not recover.	Growth of fungus out through damaged zone is accompanied by partial or complete recovery of tissues, especially in its outer region.
History of fungus after it has grown out to fresh green tissues.	Slow marginal growth until death of leaf. Invaded tissue lives and resembles earlier conditions of central region. No secondary impoverished zone beyond the new boundaries of the fungus.	Slow, meager, marginal growth accompanied by death of the tissues as soon as they are invaded. No secondary impoverished zone appears beyond the fungus.	Growth of fungus in outer tissues causes few deaths of host cells. An outer halo of impoverished tissue appears beyond the fungus, giving concentric zonation to the whole. In general, the effect of the fungus is more widespread but milder in character than in the other races.
Final diameter of fleck as seen in living tissues.	2 to 3 mm	2 mm	3 to 7 mm.
Reproductive activities.	Occasional minute uredinium—sometimes submerged.	Occasional minute uredinium. Sometimes reproductive process is begun and abandoned before spores are formed.	No spores noted. Scarcely a trace of even the beginning of uredinium formation.

One can only speculate as to the nature of this change or adjustment. From present data, one can not even be sure whether it is the fungus or the host, or both, that change. It may be that a fungus regularly secretes different substances at different periods in its ontogeny, and that the substance fatal to invaded host cells in the young infection is no longer secreted later on when host tissues survive invasion.

If the fungus does not change, the host must, for if neither had altered, and the fungous secretion continued to be as injurious to the host as it was at the outset, each host cell would die as soon as a haustorium formed in it, and the fungus would die speedily of exhaustion, for this parasite can subsist only on living tissues. But these forms live on Khapli emmer for two or three weeks.

Perhaps, on the other hand, the secretions of the young fungus set up counter-reactions in the host, by means of which later secretion of the same substance is rendered less destructive. If a host cell can protect itself against a foreign substance by neutralizing it, the cell may survive. With the host cells still living the fungus has food, and if its own metabolism is not too badly crippled by the ineffectiveness of its secretions, a new mode of living is established, in which host and fungus appear a little more congenial.

The condition suggests that obtaining in chronic bacterial infections in animals, in which (*10, p. 91*) "the body cells become accustomed, as it were, to these toxic products, and produce only sufficient antibodies to effect their immediate neutralization. The bacteria themselves become distinctly resistant to the action of the tissues and the defensive forces, and there is neither the same degree of intoxication nor reaction as are seen in acute infections."

The epidermal tissue of Khapli emmer (like that of Mindum wheat infected with form 3) shows a reaction to the fungus different from that of the mesophyll tissue. The protoplast of the invaded epidermal cell does not collapse, and the haustorium within it may attain full size. This is interesting in connection with the fact that an occasional epidermal nucleus in infected tissues of Khapli emmer undergoes extreme expansion. Evidently in these cases the outer cell layer of the leaf differs chemically from the rest and has less resistance to the disease.

Substances continue to pass from the fungus to the host. Even after the period when the host cells collapse quickly on being entered by a haustorium, there is evidence that some substance is still diffusing out into the host cell from the haustorium. This is seen in the behavior of the host nucleus, which seems to be attracted to the haustorium and moves over to it. The nucleus may squeeze in between the haustorium and the cell wall, and break the neck of the haustorium which connected it with the mother cell outside (pl. 9, D). Cases are not rare in which the nucleus and haustorium are so closely associated as to be deformed. The nucleus may be wrapped around the haustorium, or one may be cupped into the other. Apparently the nucleus is not brought passively to the haustorium by streaming cytoplasm, for there is no heaping up of cytoplasm about the haustorium. Nucleus and haustorium are commonly together in material of all ages, and this is equally true of all three forms of the fungus. Little can be inferred from this as to the nature of the fungus secretions. The results of previous workers (*3*) have shown that positive

chemotaxis of the nucleus occurs in many plants, and in response to a great variety of chemicals.

In the interchange of materials between fungus and host one or more substances diffuse from the host cell into the haustorium and on to the mother cell outside. Evidence of this is seen in the partial disintegration of the walls of the haustorium mother cells. Since, when the disintegration of fungus cellulose occurs, it is limited to the haustorium mother cell, or at farthest to the hypha below it, it is natural to suppose that the damage is due to some substance originating in the host. It can not be assumed from this, however, that Khapli can produce an enzyme capable of breaking down the very resistant walls of a fungus. Perhaps the substance from the host merely destroys or alters the permeability of the outer osmotic membrane of the fungus cell, thus exposing the fungous wall to the direct action of the enzymes normally present in the hypha.

Since this reaction is usual in form 9, much less common in form 21, and virtually absent in form 27, when the three are grown on the same host, it would appear that their respective secretions differ and that the chemical response of the host varies according to the secretion it receives.

Further evidence of differences between the three forms is to be seen in the character of the damage done in the zone of tissues surrounding the infection. In form 21 there is a rapid plasmolysis, collapse, and death of the cells. The plastids flatten, show affinity for saffranin, and later dissolve, while the nuclear net is rapidly changed into a uniform smear. This is in marked contrast to the changes in the outer affected area in infections by forms 9 and 27 on Khapli. In them the cells are not plasmolyzed, and the plastids do not change in staining reaction. The cell contents remain in place against the cell wall but become more and more meager. In some cases, especially in form 9, the cell contents disappear, leaving the empty cell wall. The latter may retain its original shape or also may disappear partly, leaving mere shreds.

In all three forms the fungus usually grows out through this damaged zone to fresh tissues beyond, but only in form 27 does this later growth result in a second impoverished zone beyond the new boundaries of the fungus.

It has sometimes been assumed that the damage done by a fungus beyond its own boundary is due to its using all the available food within its limits and draining the food from surrounding territory. Küster (*11*), in his work on zone formation in colloidal media, emphasizes the striking similarity between the Liesegang rings (formed by diffusion of silver nitrate in gelatin plates containing potassium bichromate) and the concentric growth rings of many fungi. *Actinomyces annulatus* on culture media forms definite concentric circles of conidia-bearing layers, each separated from the next by a zone of sterile mycelium. At each fruiting line the food in the substratum becomes exhausted, and the fungus must grow out radially to fresh food before fruiting again.

Also when grown on seedling leaves of a susceptible host, stem rust produces concentric rings of uredinia, each ring clean-cut and separated from the next by an area in which no spores are produced. Under favorable circumstances three and even four circles of uredinia have been noted. The outermost circles are incomplete at the sides,

for the diameter of the circle is greater than the width of the leaf. Even on a resistant host there is a recognizable minimum of zone formation.

It is open to question whether in stem rust of wheat the formation of sterile regions between fruiting layers is solely a question of food supply. The actual consumption of food by the scant mycelial growth on Khapli emmer, where few or no spores are formed, must be slight, yet the tissues immediately surrounding an infection are in worse condition than in the corresponding area on a susceptible host, where the mycelium is relatively massive. Moreover, as already stated, this outer zone presents a typically different appearance in infections by the three forms of the fungus, although the quantity of food consumed is approximately the same in all three. Furthermore, on the supposition that the damage is due to loss of food, it remains unexplained why (in a susceptible host, and even in a resistant one after the first adjustment) the cells within an infection are in better condition than the cells farther out. To be sure, the invaded cells of a resistant host die after a few days, but they remain turgid and maintain the normal form, distribution, and staining reaction of the cell contents up to the time of collapse. Nor is it clear, on the basis of food supply, why, when the fungus grows out through this outer zone, the cells, if not too enfeebled, may show a slight temporary increase in vigor.

It seems fairly certain that the fungus stimulates the cell in which it forms a haustorium, and into which it secretes materials. This stimulating effect, in the present instances at least, seems to be fairly closely confined to the cells containing haustoria, but other substances, due directly or indirectly to the fungus, diffuse out beyond, causing damage. These latter substances, judging by their effect on the tissues, are somewhat different in the three forms of stem rust considered here.

SUMMARY

The data on the three forms, 9, 21, and 27, are summarized in parallel columns in Table VI.

LITERATURE CITED

(1) Aamodt, O. S.
1923. The inheritance of growth habit and resistance to stem rust in a cross between two varieties of common wheat. Jour. Agr. Research 24: 457–470, illus.

(2) Allen, R. F.
1923. A cytological study of infection of Baart and Kanred wheats by Puccinia graminis tritici. Jour. Agr. Research 23: 131–152, illus.

(3) ———
1924. Cytological studies of infection of Baart, Kanred, and Mindum wheats by Puccinia graminis tritici forms III and XIX. Jour. Agr. Research (1923) 26: 571–604, illus.

(4) Harrington, J. B., and Aamodt, O. S.
1923. The mode of inheritance of resistance to Puccinia graminis with relation to seed color in crosses between varieties of durum wheat. Jour. Agr. Research 24: 979–996, illus.

(5) Hayes, H. K., and Aamodt, O. S.
1923. A study of rust resistance in a cross between Marquis and Kota wheats. Jour. Agr. Research 24: 997–1012, illus.

(6) Hoerner, G. R.
1919. Biologic forms of Puccinia coronata on oats. Phytopathology 9: 309–314, illus.

(7) HUNGERFORD, C. W., and OWENS, C. E.
1923. SPECIALIZED VARIETIES OF PUCCINIA GLUMARUM, AND HOSTS FOR VARIETY TRITICI. Jour. Agr. Research 25: 363–402, illus.

(8) HURD, A. M.
1923. HYDROGEN-ION CONCENTRATION AND VARIETAL RESISTANCE OF WHEAT TO STEMRUST AND OTHER DISEASES. Jour. Agr. Research 23: 373–386.

(9) HURSH, C. R.
1924. MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE RESISTANCE OF WHEAT TO PUCCINIA GRAMINIS TRITICI ERIKSS. AND HENN. Jour. Agr. Research 27: 381–412, illus.

(10) KOLMER, J. A.
1923. A PRACTICAL TEXT-BOOK OF INFECTION, IMMUNITY AND BIOLOGIC THERAPY. Ed. 3, 1210 p., illus. Philadelphia and London.

(11) KÜSTER, E.
1913. ÜBER ZONENBILDUNG IN KOLLOIDALEN MEDIEN. 111 p., illus. Jena. (*His* Beiträge zur entwicklungsmechanischen Anatomie der Pflanzen, Heft 1.)

(12) LEVINE, M. N.
1923. A STATISTICAL STUDY OF THE COMPARATIVE MORPHOLOGY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS. Jour. Agr. Research 24: 539–568, illus.

(13) ——— and STAKMAN, E. C.
1923. BIOLOGIC SPECIALIZATION OF PUCCINIA GRAMINIS SECALIS. (Abstract) Phytopathology 13: 35.

(14) LOFTFIELD, J. V. G.
1921. THE BEHAVIOR OF STOMATA. 104 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 314.)

(15) MAINS, E. B., and JACKSON, H. S.
1923. STRAINS OF THE LEAF-RUST OF WHEAT, PUCCINIA TRITICINA, IN THE UNITED STATES. (Abstract) Phytopathology 13: 36.

(16) ——— and LEIGHTY, C. E.
1923. RESISTANCE IN RYE TO LEAF RUST, PUCCINIA DISPERSA ERIKSS. Jour. Agr. Research 25: 243–252, illus.

(17) MELHUS, I. E., DIETZ, S. M., and WILLEY, F.
1922. ALTERNATE HOSTS AND BIOLOGIC SPECIALIZATION OF CROWN RUST IN AMERICA. Iowa Agr. Expt. Sta. Research Bul. 72: 211–236, illus.

(18) NEWTON, M.
1922. STUDIES IN WHEAT STEM RUST (PUCCINIA GRAMINIS TRITICI). Roy. Soc. Canada Proc. and Trans. (III) 16 (sect. V): 153–210, illus.

(19) SAX, K.
1923. THE RELATION BETWEEN CHROMOSOME NUMBER, MORPHOLOGICAL CHARACTERS AND RUST RESISTANCE IN SEGREGATES OF PARTIALLY STERILE WHEAT HYBRIDS. Genetics 8: 301–321, illus.

(20) STAKMAN, E. C., and PIEMEISEL, F. J.
1917. A NEW STRAIN OF PUCCINIA GRAMINIS. (Abstract) Phytopathology 7: 73.

(21) ——— and LEVINE, M. N.
1922. THE DETERMINATION OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON TRITICUM SPP. Minn. Agr. Expt. Sta. Tech. Bul. 8, 10 p., illus.

(22) ——— LEVINE, M. N., and BAILEY, D. L.
1923. BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON VARIETIES OF AVENA SPP. Jour. Agr. Research 24: 1013–1018, illus.

(23) ——— and AAMODT, O. S.
1924. THE EFFECT OF FERTILIZERS ON THE DEVELOPMENT OF STEM RUST OF WHEAT. Jour. Agr. Research 27: 341–380, illus.

PRACTICAL METHODS OF ESTIMATING THE PROPORTIONS OF FAT AND BONE IN CATTLE SLAUGHTERED IN COMMERCIAL PACKING PLANTS[1]

By J. L. Lush

Animal Husbandsman, Texas Agricultural Experiment Station

INTRODUCTION

This paper presents the results of an investigation which had for its object the finding of more exact practical indicators of the percentage of fat in live steers or in their dressed carcasses. The investigation was restricted to indicators which can be expressed quantitatively and are so simple and so practical that they can be applied in commercial packing plants without much trouble. Similar indicators of the percentage of bone in the dressed sides of beef would also be desirable. Data bearing upon the reliability of one such indicator are presented.

An indicator of fatness, which could be expressed quantitatively and which would be free from the personal opinion of the men who judge the live cattle and the dressed meat, would be very helpful in interpreting the results of feeding trials. As far as fatness is concerned, the usual practice in publishing the results of a cattle-feeding trial or series of trials is merely to give the prices for which the live cattle were sold and perhaps also their dressing percentages and a few comments by the men who bought them. Sometimes the investigator goes so far as to analyze a wholesale cut or a few slices of beef, (*6*, *10*),[2] but the procedure is far from standardized.

A quantitative measure of the difference in the fatness of two lots is especially desirable in feeding trials designed to test out the productive value of different rations. This is due to the fact that a pound of true fat gained by the animal requires for its production several times as much net energy from the ration as does a pound of nonfat live weight. If the investigator does not have any estimate of the proportion of true fat included in the live-weight gain made by different lots he is handicapped in his interpretation of the results.

There probably does not exist any quantitative indicator or combination of indicators of fatness so accurate as to enable investigators to discard all personal opinions from the experimental data. Nevertheless, the investigator who uses a good quantitative indicator to supplement his own judgment and that of the cattle buyer, even though the indicator is not perfect, is less liable to draw erroneous conclusions from his feeding trials than if he relies upon human judgment alone. A definite knowledge of the degree of fatness of the dressed meat would add much to the knowledge of its value for human food, although it may be true, as maintained by Moulton and his coworkers (*15*), and by Mackenzie and Marshall (*10*), that the desirability of fatness has been overemphasized in much of the literature on the subject.

[1] Received for publication Aug. 28, 1925; issued March, 1926.

[2] Reference is made by number (italic) to "Literature cited," p. 754.

A knowledge of the percentage of bone in dressed beef would be of value chiefly in tests of different ages, types, or breeds of cattle. It might have some value in feeding trials where minerals were used. It would also occupy a prominent position in all studies of the quality and food value of the meat. This knowledge can be obtained by boning the meat, but this method is expensive.

Information for this study was collected from all sources which had published data pertinent to this problem.

DATA STUDIED

By the very nature of the problem, data which could be used were restricted to those experiments in which cattle were so carefully slaughtered that the weight of each part was accounted for, and in which the composition of each part (at least with respect to fat) was determined by chemical analysis. Four groups of published data which could be used were found. They were the analyses of 30 steers and 3 cows made at the Missouri Agricultural Experiment Station (*11*, *12*, *13*), the analyses of 4 steers made at the Maine Agricultural Experiment Station (*8*), the analyses of 3 steers made at the Illinois Agricultural Experiment Station (*4*), and the well-known analyses of 2 oxen and a calf made by Lawes and Gilbert (*9*) in England nearly 70 years ago. The extensive analyses of steers made at the Minnesota station (*3*) could not be used because the individual organs were not weighed and analyzed separately. It was thought possible that combining data from two or more different investigators might lead to erroneous conclusions on account of differences in the technique of slaughter and analysis. Therefore, wherever a plotting of the data showed a possibility of disagreement two calculations were made, one including all the cattle analyzed and the other including only the largest single group, the 30 steers of beef type analyzed at the Missouri station. It is for this reason that the source of each analysis is indicated by the symbols used in the graphs. It was also thought possible that the relations which are true for cattle of one age might not be true for cattle of another age. Therefore the 30 steers analyzed at the Missouri station are shown in the graphs by one or the other of two different symbols, according to whether they were over or under 1 year of age when slaughtered. Parenthetically, it should be observed that the data reveal no difference between the older and younger cattle from the point of view taken in this study, except the well-known fact that younger cattle contain a smaller percentage of true fat than older cattle, and possibly a difference in the slope of the regression equations for fatness and dressing per cent, and in the slope of the regression equations involving percentage of bone in the dressed meat.

It was necessary to calculate some of the percentages which were not calculated in the original publications and to recalculate others which were not presented in a comparable form in the material from all four sources. Thus it was necessary to calculate the percentage of bone in the dressed carcasses of the steers slaughtered at the Maine station, and there were two methods of doing this which did not give exactly identical results. Those results were used which seemed, from the context of the article in which they were presented, to have been obtained in a manner most nearly comparable to that

used at the Missouri station. Also, the basis for figuring percentages was the live weight of the animal instead of the "warm empty weight" used in presenting the data taken at the Missouri station. This was done because the "warm empty weight" can not be determined on the floor of a commercial packing house. Therefore "warm empty weight," in spite of the advantages of using it in some studies, is practicable only in experimental abattoirs, where very few cattle are killed at a time and where there is no pressing need for haste. The warm weights of the carcasses were used instead of the chilled weights, since it is the warm weights which are actually obtained in the commercial packing plants as a basis for further figures. The "chilled weight" of ordinary commercial practice is not an actual weight at all, but is some arbitrary fraction of the warm weight, usually 97.5 or 98 per cent. The percentage of fat in the entire live animal in the Maine data had to be calculated on the assumption that there was no fat in the skin or in the contents of the digestive tract, an assumption which is not absolutely accurate, but is so nearly so that it has very little effect upon the results. The percentage of fat in the entire live animal was not given in the Illinois data, but was calculated from the percentage of fat in the boneless meat from the dressed sides. The high correlation which seems to justify this calculation is discussed later in the section on "Relation of true fat in entire animal to true fat in lean and fat flesh." The figures actually used are presented in Table I. It is believed that they are accurate and comparable to a high degree. However, there may be several other very minor discrepancies, such as the length of time feed was withheld from the animals before slaughter. This was not stated for the Maine data, but would influence the live weight and all percentages derived from it. The time between the last feeding and slaughtering was longer in the case of the Lawes and Gilbert steers than in the case of the Missouri cows and steers.

RELATION OF TRUE FAT IN ENTIRE ANIMAL TO TRUE FAT IN LEAN AND FAT FLESH

From the standpoint of studying the quality of meat and its usefulness for food, the percentage of fat in the lean and fat flesh (i. e., in that part of the dressed carcass which would be left after a butcher had cut all the bones out of it) is more important than the percentage of fat in the entire live animal. From the standpoint of interpreting the results of feeding experiments, it is the percentage of fat in the entire live animal which is the more important. Therefore the first step in the search for indicators of fatness was to compare the percentage of fat in the entire live animal with the percentage of fat in the lean and fat flesh of the same animal, to see whether these two percentages were so highly correlated that the same indicator would serve for both purposes. A fairly high degree of correlation between these two percentages is to be expected, because they are to a large extent determined by common factors. Thus, the percentage of fat in lean and fat flesh is $\frac{M}{M+A}$, and the percentage of fat in the entire live animal is $\frac{M+N}{M+A+N+B}$, where

M equals weight of fat in lean and fat flesh,
A equals weight of nonfat material in lean and fat flesh,
B equals weight of nonfat material in bones and noncarcass parts,
N equals weight of fat in bones and noncarcass parts.

M is much larger than N; and in many cattle, particularly in fat cattle, $M+A$ is larger than $N+B$. Therefore variations in $\frac{M}{M+A}$ would be accompanied by corresponding variations in $\frac{M+N}{M+A+N+B}$, even if N and B were not correlated at all with M and with A, respectively. If, as is much more likely to be the case, M is highly correlated with N and A is highly correlated with B, then the correlation between the two percentages which are formed from A, B, M, and N would be high indeed.

TABLE I.—*Percentages of fat and bone in individual animals*

Animal No.	Percentage of true fat in entire live animal	Percentage of true fat in lean and fat flesh	Percentage of offal fat to live weight	Percentage of caul fat to live weight	Dressing per cent	Percentage of true fat in offal fat	Percentage of true fat in kidney fat	Percentage of leg bones to live weight	Percentage of bone in dressed carcass
Jersey 4	26.20	33.42	7.30	2.42	55.91	90.53	96.28	1.10	13.99
Jersey 43	24.83	29.73	8.17	2.31	53.77	88.20	94.38	1.06	13.81
Jersey 63	17.58	23.35	3.63	1.06	54.69	82.18	91.22	1.29	16.83
500	15.01	17.23	2.83	.86	59.36	83.56	90.28	1.88	20.36
501	40.18	53.12	4.37	1.66	68.95	91.06	93.31	1.13	10.22
502	14.53	18.22	2.24	.54	60.28	86.18	89.67	1.73	18.07
503 [a]	14.01	18.39	2.73		54.05	81.92	89.47	2.09	20.21
504	25.99	35.13	4.77		58.24	84.82	93.94	1.35	13.70
505 [a]	21.38	29.68	4.08		56.45	85.38	93.53	1.58	15.98
507	17.19	21.48	2.47	.84	60.49	83.96	91.30	1.68	18.00
509	14.25	16.96	2.26	.67	60.34	85.89	92.41	1.72	18.75
512	21.35	28.29	3.18	1.01	61.80	86.27	93.92	1.54	18.69
513	37.78	49.37	6.30	2.88	65.32	92.87	94.93	1.12	11.00
515	32.15	45.22	4.02	1.25	61.49	90.29	94.43	1.31	13.01
523	12.77	16.50	2.08	.57	57.71	81.28	92.73	1.71	17.33
524	9.07	8.86	1.38	.29	58.08	69.44	76.83	2.03	23.43
525	9.87	11.91	1.63	.37	54.27	70.64	90.18	1.76	19.52
526	17.56	22.38	2.41	.58	61.18	84.14	87.83	1.61	18.32
527	41.55	51.80	5.76	1.69	70.42	93.26	93.23	1.09	9.94
531	8.99	10.03	1.36	.32	56.02	70.68	90.27	2.10	20.69
532	23.08	28.72	4.58	1.69	60.46	88.81	95.23	1.49	15.54
538 [a]	11.63	14.01	1.91	.52	53.66	76.45	90.96	2.07	20.42
540 [a]	10.03	11.72	1.46	.37	55.23	71.06	79.81	2.05	21.64
541 [a]	18.19	23.09	3.19	1.30	58.59	86.40	94.35	1.63	14.96
547 [a]	13.00	17.92	1.88	.54	54.17	78.88	90.10	2.14	17.79
548 [a]	5.79	4.73	.85		54.39	36.78	64.22	2.88	26.54
550 [a]	11.49	14.30	1.77	.36	50.46	74.69	86.88	2.14	22.86
552 [a]	8.83	9.45	1.53	.31	53.00	67.76	84.56	2.84	24.49
554 [a]	6.46	5.94	.74	.19	57.32	50.87	75.42	3.09	27.60
555 [a]	3.62	3.26	.55	.13	53.63	31.37	57.41	2.87	27.34
556 [a]	8.35	9.14	1.26	.37	54.19	61.31	80.62	2.64	25.53
557 [a]	17.52	24.82	3.30	.97	53.71	83.47	89.77	2.02	19.39
558 [a]	7.02	5.52	.77	.14	51.09	43.21	75.16	2.75	27.50
Lawes and Gilbert fat calf	14.60		2.74	.96	62.05	77.36		1.70	14.79
Lawes and Gilbert, half-fat ox	18.70		3.14	1.35	64.75	84.68		1.63	13.12
Lawes and Gilbert moderately fat ox	30.00		5.14	2.10	66.20	95.03		1.58	11.80
Jordan's 1	14.42	18.44	2.33		57.75	[b] 86.90	96.17	1.93	12.98
Jordan's 2	19.61	25.03	3.11		56.96	[b] 91.28	94.82	1.76	14.86
Jordan's 3	18.56	25.13	2.70		57.23	[b] 89.68	94.86	1.61	16.27
Jordan's 4	15.95	20.06	2.29		59.06	[b] 85.74	93.06	1.79	14.13
Illinois Hereford	[c] 25.21	32.84	5.15	1.77	60.86			1.59	13.16
Illinois Angus	[c] 24.73	32.17	5.97	2.47	62.10			1.26	12.14
Illinois Shorthorn	[c] 29.98	39.46	4.71	1.80	63.97			1.38	11.73

[a] Under 1 year of age. [b] Intestinal. [c] Estimated.

The actual relation between the two percentages is shown graphically in Figure 1. The relations are linear and the coefficient of correlation is $+0.9950 \pm 0.0012$ when only the 30 steers of beef type slaughtered at the Missouri station are considered, and the coefficient is $+0.9944 \pm 0.0012$ when the 3 Jersey cows slaughtered at the Missouri station and the 4 Shorthorn steers slaughtered at the Maine station are included. When it is borne in mind that such errors as occurred in the technique of analysis more probably lowered rather than raised the coefficient of correlation from its true value (on account of its already extremely high value), it is quite reasonable to conclude that M is correlated with N and that A is correlated with B to an extraordinarily high degree, and that we are here dealing with two measurements of what is essentially a single characteristic, the fatness of the animal. This simplifies the search for a reliable indicator of fatness, since the same indicator will serve in

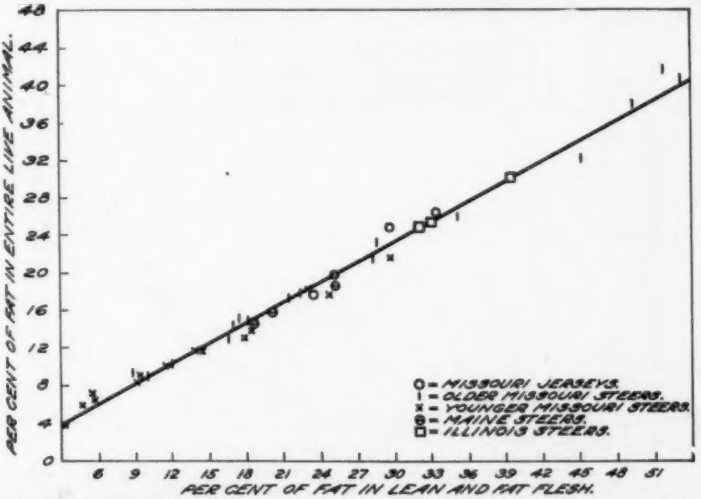


FIG. 1.—The relation between percentage of true fat in entire live animal and percentage of true fat in lean and fat flesh. The straight line is the equation, $y = 0.7197x + 1.58$ per cent, and is derived, by the method of least squares, from all the data shown in the figure except those for the Illinois steers

investigating the fatness of the meat and in interpreting the results of the feeding experiment. Because these two percentages are so perfectly correlated it will be unnecessary to consider both of them further, and therefore only the percentage of fat in the entire body will be used further in this paper as a criterion with which to test the accuracy of possible indicators of fatness.

Before leaving this subject, however, it may be well to give the regression equations by which one of these percentages may be predicted when the other is known. These equations, based upon all 37 animals, are as follows:

(1) Per cent of fat in entire body, 0.7197 times the per cent of fat in lean and fat flesh + 1.58 per cent.

(2) Per cent of fat in lean and fat flesh, 1.3740 times the per cent of fat in entire body − 1.92 per cent.

Equation No. 1 was used in calculating the percentage of fat in the entire live animal in the case of the 3 Illinois steers.

PER CENT OF TRUE FAT IN FATTY TISSUE AS AN INDICATOR OF THE FATNESS OF THE ENTIRE ANIMAL

It is a matter of common knowledge that the material which the butcher calls fat is not all fat in the chemical sense of the word, but contains a varying amount of moisture and small amounts of protein and ash, and that the per cent of true fat in fatty tissue increases as the animal becomes fatter, and that percentages of the other constituents of the fatty tissue decrease. Especially is this true of the percentage of water in fatty tissue. Such common trade terms as "washy" and "lacking in substance," which are frequently applied to immature grass-fed cattle marketed early in the season, are a practical recognition of this condition.

It was thought possible that this increase in the percentage of fat in fatty tissue might be sufficiently regular with the progression of the fattening process to be useful as an indicator of the fatness

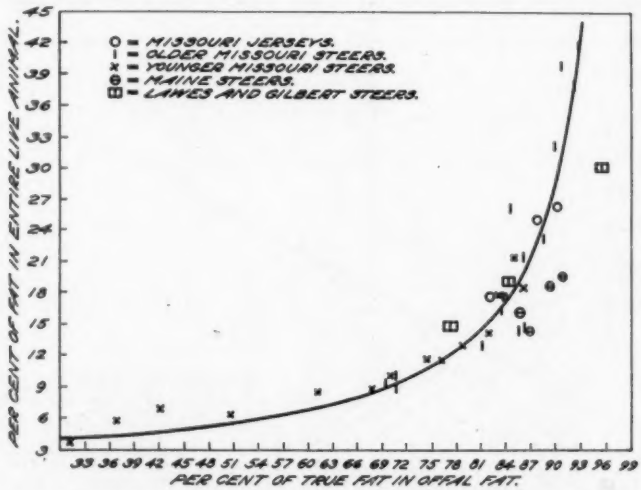

O = MISSOURI JERSEYS.
I = OLDER MISSOURI STEERS.
× = YOUNGER MISSOURI STEERS.
⊖ = MAINE STEERS.
⊟ = LAWES AND GILBERT STEERS.
PER CENT OF FAT IN ENTIRE LIVE ANIMAL.
45 42 39 36 33 30 27 24 21 18 15 12 9 6 3
33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99
PER CENT OF TRUE FAT IN OFFAL FAT.

FIG. 2.—The relation between percentage of fat in entire live animal and percentage of true fat in offal fat. The curve is the equation, $xy - 100y = -272.5$, and is based on all the data shown

of the entire animal. If this were so it would be a very easy matter to get a measure of fatness by taking samples of offal fat or kidney fat from each animal on the killing floor of the packing plant and analyzing those samples for fat later.

The relation between the percentage of true fat in offal fat and the percentage of fat in the entire animal is shown in Figure 2. The similar relation between the percentage of true fat in kidney fat and the percentage of fat in the entire animal is shown in Figure 3. There is a very definite and close relationship between each of these two possible indicators and the percentage of fat in the entire animal, but the relationship is not at all a straight line, and therefore the ordinary methods of correlation are not applicable. It is evident that these indicators have little value as such for either extremely thin or extremely fat cattle, since in the extreme ranges of fatness one of the variables changes but slightly with a large change in the other.

In order to express these relationships as definitely as possible, an attempt was made to find the equation of the curve which most nearly fits the data in Figure 2 and in Figure 3. Figure 2 is very closely fitted by the rectangular hyberbola $xy-100y=-272.5$, which is shown in the figure. Figure 3 is only fairly well fitted by its rectangular hyperbola $xy-100y=-146.4$, which is also shown. The chief discrepancy between the equation and the data is that the percentage of fat in kidney fat seems to approach as a limit, not 100 per cent, but about 95 or 96 per cent. This discrepancy indicates a continued increase in the nonfat part of fatty tissue, even in extremely fat animals. Perhaps this can be more clearly presented by the following formula:

If P equals the weight of fat in kidney fat, and
C equals the weight of nonfat in kidney fat, then
$\frac{100\,P}{P+C}$ equals per cent of fat in kidney fat.

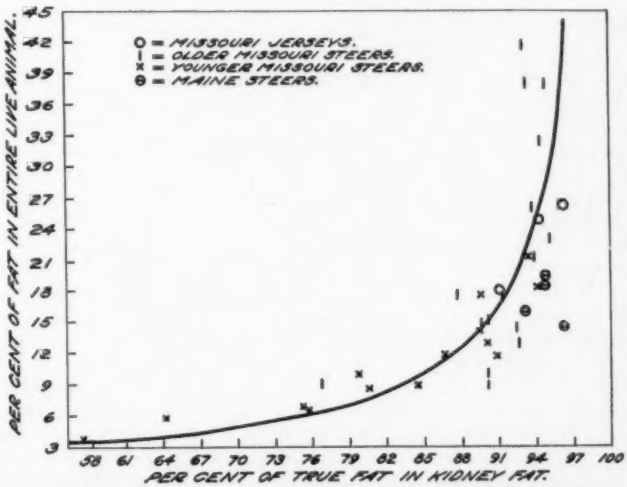


FIG. 3.—The relation between percentage of fat in entire live animal and percentage of true fat in kidney fat. The curve shown is the equation, $xy-100y=-146.4$, and is based on all the data shown

In the early stages of fattening the percentage increases in P are relatively enormous compared to the percentage increases in C, and therefore the value of $\frac{P}{P+C}$ rises very rapidly. If C did not increase at all $\frac{P}{P+C}$ would approach 1.00 as a limit, but if C continues to increase also, even though very slowly, $\frac{P}{P+C}$ will approach as a limit a value somewhat less than 1.00. The exact limit will be reached when $\frac{P}{C}$ equals $\frac{\Delta P}{\Delta C}$, and one least-squares solution of the present data for a rectangular hyperbola gives that limit as $\frac{100\,P}{P+C}$

equals 94.8, but inspection of the data would seem to indicate that the true physiological limit of fatness of fatty tissue is slightly higher than that.

These figures and equations show that in the early stages of fattening the fatty tissue changes in composition more than in quantity, but that as fattening progresses the composition tends to reach its physiological limit of fatness, and thereafter fatty tissue increases in quantity but remains fairly constant in composition. No difference in this behavior is shown by cattle of different ages and breeds. In the same animal kidney fat contains a higher proportion of true fat than offal fat does.

While these relations have a great deal of interest for physiology and nutrition, the shape of the curves is such that the percentages of fat in offal fat or in kidney fat can not be used as indicators of the per cent of fat in the entire live animal except within wide limits, and therefore they will not be considered further in this paper.

DRESSING PER CENT AS AN INDICATOR OF THE PER CENT OF FAT IN THE ENTIRE LIVE ANIMAL

Dressing per cent as an indicator of fatness in a general way has been widely used. The report of a feeding experiment is considered hardly complete without a statement of the dressing per cents of the various lots. However, dressing per cent has other significance than merely as an indicator of fatness, for upon it depends very largely the margin which the packer must receive between the price of live cattle and the price of dressed beef before he can show a profit. It is in this significance that dressing per cent is most commonly used, especially by men who work in the terminal stockyards and packing plants. However, it is nearly always inferred in such usage that the higher dressing per cent is associated with the fatter animal, and that is undoubtedly true where large enough numbers of cattle are compared unless the cattle differ in sex, breed, or general type. However, so many things other than fatness are thought to affect dressing per cent that, so far as the writer is aware, it has not been used in the interpretation of feeding experiments except in a most general way. Indeed, the practice of figuring dressing per cent is not uniform with respect to whether it is figured on the basis of final feed-lot weights (so as to be more comparable with other experimental results) or on the basis of market live weights (so as to be more comparable with commercial cattle). Dressing per cent is usually figured on the basis of warm dressed weights, from which a certain percentage (usually 2.5 per cent) is deducted to allow for the loss in weight during chilling.

The data upon which this paper is based were figured from the warm dressed weight of the carcass and the live weight of the animal just before slaughter. The animals were slaughtered close to where they had been fed, and there was no shrinkage in transit to be accounted for. The relation between dressing per cent and the per cent of fat in the entire body is shown in Figure 4. Differences in the length of time food was withheld before slaughter may account for the unusually high dressing per cent of the Lawes and Gilbert steers. The unusually low dressing per cent of the two fat Jersey cows may be evidence of a breed or sex difference.

It will be seen from inspection of Figure 4 that in a general way fatness and high dressing per cent are correlated, but that there is a great deal of variation, and when one considers only animals within a narrow range of fatness dressing per cent is not a very reliable indicator of fatness. The correlation between dressing per cent and per cent of fat in entire live animals is $+0.760 \pm 0.043$ when all 43 animals are considered, and is $+0.842 \pm 0.036$ when only the 30 beef steers slaughtered at the Missouri station are considered.

This latter figure excludes the effects of differences in slaughter technique and also of breed differences, and therefore probably expresses the maximum degree of correlation between these two variables under all the most favorable conditions (except uniformity in age) to show a high correlation. Using it as the more nearly correct of the two, the standard error of estimate [3] becomes only 53.9 per

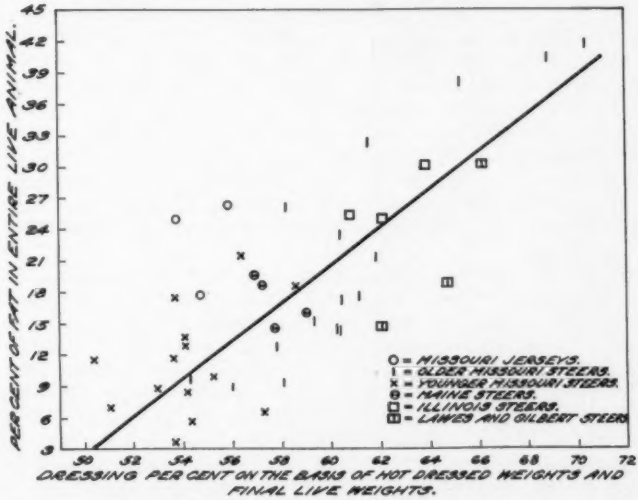


FIG. 4.—The relation between percentage of fat in entire live animal and the dressing per cent. The straight line is the equation, $y = 1.782r - 86.40$, and is based on the data for the 30 Missouri steers

cent as large as the standard deviation, and the regression equation for predicting the amount of fat in the entire live animal from the dressing per cent is:

> Per cent of fat in entire live animal = 1.782 times the dressing per cent − 86.40 per cent.

In applying this equation to other experimental cattle the conditions should as closely as possible duplicate those of the Missouri experiments, i. e., dressing per cent should be figured on the basis of final feed-lot weights and warm-carcass weights. The difference between final feed-lot weights and stockyards weights, commonly known as shrink, is probably mostly due to losses in the contents of the digestive tract. If it were entirely due to this the use of final feed-lot weights would be exactly comparable to the Missouri conditions. However,

[3] For a brief exposition of the derivation and meaning of the "standard error of estimate" and other statistical terms used in this paper, the reader is referred to "Correlation and machine calculation," by H. A. Wallace and George W. Snedecor (*16*).

a small part of the shrink may be due to losses in actual flesh, and to the extent that this is so the use of final feed-lot weights for figuring percentages will give lower values than if the animals had been slaughtered under exactly similar conditions to those in the Missouri experiments. The losses in actual flesh are probably small because the daily maintenance requirement for a 1,000-pound steer is equivalent in energy to only about 1½ pounds of true fat, and few experimental cattle are longer than two days in transit. Even if the maintenance requirement is multiplied several times to allow for the work and nervous excitement of the cattle during shipment, the amount of actual flesh lost would still be a relatively small proportion of the shrink. Therefore final feed-lot weights seem to be more nearly accurate for this purpose than stockyard weights, although it should be kept in mind that the use of final feed-lot weights will probably result in percentages slightly too low. In cases where cattle are kept in the stockyards long enough to completely recover their normal feeding habits the use of stockyard weights would probably be more accurate. In actual practice, however, this would be confined to show cattle which had been kept fully supplied with feed for at least four or five days after arrival in the stockyards.

There is also an indication in these figures that with older steers small differences in dressing per cent mean larger differences in fatness than with younger steers. Figure 4 shows this graphically, and raises the question as to whether the use of this equation would not give values too low for the fatness of older steers which were in good condition.

Murray (*14*) estimates that animals which the practical expert would describe as "fair," "half fat," "moderately fat," and "fat" all come within the range of containing from 15 per cent to 35 per cent of true fat in their live weight. If all animals outside those limits were excluded from Figure 4 the correlation would be much lower than it is for the entire group. In other words, dressing per cent is not nearly so reliable an indicator of fatness as the above figures would indicate when the range of fatness is restricted. And yet a range of from "fair" to "fat" is a wider range than would be encountered in any ordinary group of experimental cattle.

It seems clear, then, that dressing per cent is a good general indicator of fatness, but is affected by too many other things to be very trustworthy, particularly if the range of fatness in the group of animals to be studied is small. This conclusion is in agreement with the results of such other investigations of the question as have come to the writer's attention. Thus Hammond (*5*) summarizes the work of Mackenzie and Marshall (*10*) in regard to this point as follows: Mackenzie and Marshall found, however, that while generally speaking a high carcass percentage is correlated with high fat content and with a low percentage of cartilage, bone, and lean meat, yet there were many and marked individual variations from this rule.

Mackenzie and Marshall (*10*) did not analyze for fat the entire steers with which they worked. Instead they analyzed the seventh rib cut, and did not include in their figures (for per cent of fat) the fat which was scattered about within the muscles, i. e., the marbling fat. From their table the writer has calculated the coefficient of correlation between dressing per cent and per cent of fat in the seventh rib cut. This coefficient is $+0.63 \pm 0.04$ for the group of

84 steers with which they worked. From this rather low correlation it would seem that the untrustworthiness of dressing per cent as an indicator of fatness, especially within narrow ranges of fatness, has not been exaggerated in the preceding statements.

PER CENT OF OFFAL FAT TO LIVE WEIGHT AS A MEASURE OF FATNESS

The per cent of offal fat to live weight is very easily obtained for a group of cattle, but the obtaining of this percentage for each animal individually would interfere very considerably with the routine of packing-house operation. The packers themselves obtain it for each group of cattle which they slaughter, merely as a matter of interdepartmental bookkeeping in order to figure the beef cost of each lot. The two largest pieces of the offal fat are the caul and the ruffle (mesentery), but these two together constitute less than half of the total offal fat, which includes many small bits of fat from the viscera. The caul can be weighed separately for each animal without much extra trouble, but the weighing of the other offal fat for individual animals involves the stripping of the intestines separately and is hardly practicable. Even weighing the ruffle for individual animals is considerable trouble, since it involves tagging each set of intestines and following them to another part of the packing plant to get the weights. Hence the per cent of offal fat to live weight is easy to get for groups, but if indicators for individual animals are desired about all that can be obtained is the weight of the caul and the ruffle.

It is, or was until recently, the commercial practice among many packing plants not to weigh all the offal fat from each lot separately, but rather to weigh the caul fat and the ruffle fat from each lot separately and to figure the total edible noncarcass fat for each lot by some such formula as the following:

Total edible noncarcass fat = 2.5 (caul plus ruffle) 2 pounds for each animal. This formula is said to have checked up well with the actual weights of fat obtained in each day's kill in a commercial packing plant with which the writer is quite familiar, and to have been abandoned only because the actual weights of the different classes of fats lent themselves much better to the requirements of interdepartmental bookkeeping. It is also said, however, that neither the caul nor the ruffle could be used alone in any formula, because the proportion in which these two stood to each other was so much affected by sex, age, condition, and type of the animals.

The Institute of American Meat Packers was asked how generally the formula quoted above was used in American packing plants. The following is quoted by the institute as a representative reply to the inquiries which it made of the different companies as to how they figured the fat yield of a lot of cattle:

> We can identify the caul fat and ruffle fat by lots, but we can not identify the gut fat, or, as some packers term it, bulk fat, so we get the actual weight of the caul and ruffle fat and from this determine the weight of the gut fat.
>
> Different packers have various methods of arriving at the weight of the gut fat. We consider the percentage basis the best method, as it gives us a chance to check our estimates and by so doing we can not get very far out of line. Our method is as follows: On good cattle, with caul and ruffle fat weighing 40 pounds or over per head, we estimate the gut fat as equal to 100 per cent of the combined weight of the caul and ruffle fat, and as the weight of the caul and ruffle

decreases per head we increase the per cent of gut fat. This is on the basis that the poorer grades of cattle would not have as heavy gut fat as the good cattle, but the gut fat would be heavier in proportion to the caul and ruffle fat.

At the end of the day's business an actual weight of the gut fat is obtained in total, and this weight compared with the total estimated weight that was used on the tests tells how close the percentage scale is running to the actual. If the estimated weight is running under the actual weight of the gut fat, then the percentage scale should be raised. If the estimated weight is running heavier than the actual weight, then the percentage scale should be lowered. In this way the estimated weight will keep fairly well in line with the actual weight, and would never get out of line to any great extent.

Since these methods of estimating the total offal fat are all empirical and vary at different plants and with different grades of cattle, it would seem that the best practice for the investigator to follow (in experiments in which he does not attempt any further analysis of the dressed meat) would be to use the empirical formula actually used in that particular plant for that particular grade of cattle.

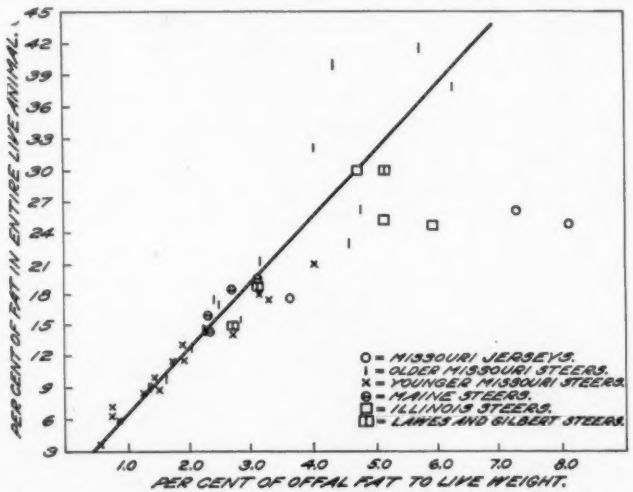


FIG. 5.—The relation between percentage of fat in entire live animal and percentage of offal fat to live weight. The straight line is the equation, $y=6.304x+0.30$, and is based on the 30 Missouri steers only

However, if he will also state the exact weight of the caul and ruffle fat from his cattle his results can be more accurately compared with other results obtained at other packing plants and in other years.

The relation between the percentage of offal fat and the percentage of fat in the entire live animal is shown in Figure 5. It will be seen that the correlation is extremely high, except for the fat Jersey cows (which have a very heavy offal fat) and for a slight tendency among the extremely fat steers for the offal fat not to continue to increase quite as fast as the fat in the entire body. The Illinois steers (which were of three different breeds) also differ somewhat from the other steers. This is especially true of the Aberdeen Angus steer, and perhaps emphasizes the need of caution in using this indicator of differences in fatness between cattle of different breeding.

The coefficient of correlation when the entire 43 head are considered is $+0.838 \pm 0.031$, and is $+0.938 \pm 0.015$ when only the 30 beef-bred steers from the Missouri station are considered. Since the latter figure largely excludes breed differences and differences in slaughter technique, it probably represents more correctly the facts as they exist in an ordinary feeding experiment where there is no breed difference and the animals are supposed to differ only in age or in the ration which they receive. Using the figures for the 30 Missouri steers, the standard error of estimate becomes only 35 per cent as large as the standard deviation, and the equation for estimating fatness becomes: Per cent of fat in entire live animal $= 0.30 + 6.304 \times$ percentage of offal fat to live weight.

The constant in this equation (0.30) is so small that it can almost be neglected, and for most purposes it is sufficiently accurate to say that the offal fat varies directly with the percentage of fat in the entire animal.

Thus the percentage of offal fat to live weight is a much more reliable measure of fatness than is dressing percentage alone, having reduced the standard deviation 65 per cent as compared with a reduction of 46 per cent which is accomplished when dressing per cent is used as an indicator. Again, however, it is necessary to use caution in interpreting the results. For instance, in comparing heifers with steers there may be a sex difference in percentage of offal fat which does not parallel differences in fatness. Thus Hammond (*5*), who studied the records of the Smithfield contests, writing of "suet fat" says: "The results confirm the generally well-known fact that heifers have more internal fat than steers." However, speaking later of "gut fat" he says: "With gut fat, conditions are reversed and the steer carries a slightly larger proportion than the heifer." Since the entire animals were not analyzed at Smithfield, it would perhaps be premature to say whether heifers really do have a higher percentage of internal fat than steers of the same degree of fatness, or whether the heifers may not actually contain a larger percentage of fat in their entire bodies than steers which appear to the stock judge to be of about the same degree of fatness. Or Hammond may be speaking here of exactly the same thing to which the practical packing-plant men referred when they said that neither caul fat nor ruffle fat alone could be used to estimate total offal fat when different kinds of cattle were compared, but that a combination of the two could be used, because each neutralized the defects of the other. At any rate it is necessary to use caution in applying any of these indicators to cases where the experimental cattle are known to differ in anything except age or the feeding treatment they received previous to slaughter.

PER CENT OF CAUL FAT TO LIVE WEIGHT AS AN INDICATOR OF FATNESS

Aside from the warm-carcass weight (from which an individual dressing per cent may be calculated), the only promising data on fatness which can be obtained without much trouble for individual animals killed in a commercial packing plant are the weight of the caul fat and (with a little more trouble), the weight of the ruffle fat. It seemed worth while to inquire into the reliability of the weight of the caul fat as an indicator of fatness, because in some feeding experiments, particularly those in which correlation studies are made of

body measurements with rates of gain and fatness, it is highly desirable to have a reliable measure of the fatness of each individual animal and not to be restricted to a knowledge of the average fatness of the whole lot.

The relation between the percentage of caul fat and the percentage of true fat in the entire live animal is shown in Figure 6. There is a very close relation, except for the fat Jersey cows from Missouri and the Angus steer from Illinois. Also, the extremely fat animals show more variation than the thin ones. The distribution is in every way similar to that of the percentage of total offal fat, except that the percentage of caul fat is not quite so regular in its relation to the percentage of fat in the entire animal. The weight of caul fat was not given for 4 of the Missouri steers or for the Maine steers. The coefficient of correlation for the entire 35 animals shown in Figure 6 is $+0.845 \pm 0.033$, and for the 26 Missouri steers it is $+0.892 \pm 0.027$. It is thus evident that the percentage of caul fat is almost but not quite as good an indicator of the fatness of an animal as is the percentage of total offal fat.

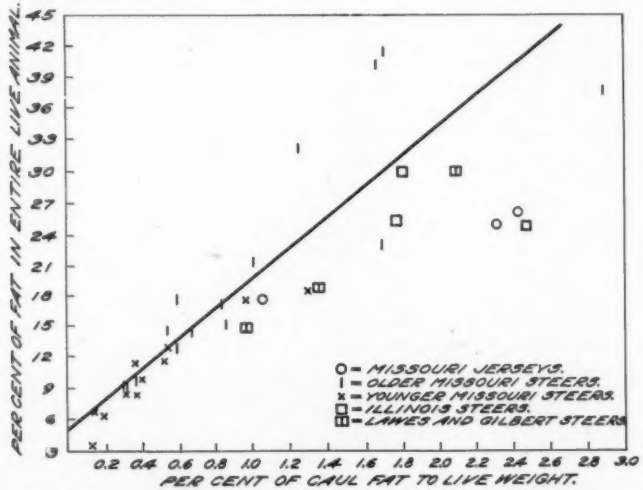


FIG. 6.—The relation between the percentage of fat in entire live animal and percentage of caul fat to live weight. The straight line is the equation, $y=14.55x+5.17$, and is based on the data for the 26 Missouri steers only

The figures for ruffle fat by itself or combined with caul fat were not given in the data used in this study, and hence the figures can not be given to test the accuracy of the statement by the practical packing-plant men that the combined weight of caul and ruffle are more accurate indicators of total offal fat than is caul alone. However, unpublished data of the Texas station on groups of steers of similar breeding and feeding show a high degree of correlation between the weights of caul fat and ruffle fat, and since the two together make up a very large part of the total offal fat, it is scarcely possible that the correlation of the combined weights of caul and ruffle with total offal fat could be as small as the correlation of the weights of caul fat alone with total offal fat.

Using the figures for the 26 Missouri steers as being the most comparable to conditions ordinarily encountered in a feeding experiment, the equation for estimating fatness from the percentage of caul fat to live weight is:

Percentage of fat in entire live animal = 5.19 per cent + 14.55 × percentage of caul fat to live weight.

The fact that the constant in this equation (5.19 per cent) is of considerable size agrees well with that practical formula of the packing plant in which 2 pounds per animal was allowed for edible noncarcass fat in addition to whatever was calculated from the weights of the caul and ruffle.

USE OF A COMBINATION OF DRESSING PER CENT AND PER CENT OF OFFAL FAT AS AN INDICATOR OF FATNESS

Of the indicators considered so far it has been shown that for groups of cattle the per cent of offal fat to live weight is the most reliable indicator of fatness, and dressing per cent is also valuable although not nearly so reliable. A combination of the two should prove more reliable than either one alone. Using all 43 animals, the correlation between dressing per cent and per cent of offal fat is $+0.472 \pm 0.080$, and the coefficient of multiple correlation of per cent of fat in the entire body with dressing per cent and per cent of offal fat is $+0.934$, which shows that a combination of the two indicators is a very distinct improvement over either one alone. Using only the 30 beef-bred Missouri steers, the coefficient of correlation between dressing per cent and per cent of offal fat is $+0.728 \pm 0.058$, and the coefficient of multiple correlation of per cent of fat in the entire body with dressing per cent and per cent of offal fat is $+0.966$, which is only a slightly higher coefficient than the simple correlation coefficient between per cent of fat in the entire animal and the per cent of offal fat. Since the addition of dressing per cent to per cent of offal fat improved the accuracy of the indicator so much in the case of the entire 43 head, but only slightly in the case of the 30 head of fairly uniform breeding which were slaughtered under uniform conditions, it seems probable that one or both indicators are affected very much by differences in breed, sex, and differences in slaughter technique. Therefore dressing per cent should always be included when the comparison is between cattle slaughtered at different places or at different times, but is not so necessary when the cattle being compared have all had the same shipment conditions and the same treatment in slaughtering. However, the inclusion of dressing per cent adds a little to the accuracy even under the most uniform conditions, and for the sake of completeness it should always be given, even though it may not be used as an indicator of fatness. Using Wright's (*17*) path coefficients to form a score card having as its object the most accurate prediction of fatness from a knowledge of dressing per cent and per cent of offal fat, the score card where all 43 animals are considered is:

	Per cent
Dressing per cent	43. 2
Per cent of offal fat	56. 8
Total	100. 0

and the standard error of estimate is only 35.7 per cent as large as the original standard deviation. The multiple regression equation is:

Per cent of fat in entire animal = 3.130 (per cent of offal fat) + 0.940 (dressing per cent) − 46.52 per cent.

Where only the 30 beef-bred Missouri steers are used the score card is:

	Per cent
Dressing per cent	32. 9
Per cent of offal fat	67. 1
Total	100. 0

and the standard error of estimate is only 25.8 per cent as large as the original standard deviation. The multiple regression equation is:

Per cent of fat in entire animal = 4.65 (per cent of offal fat) + 0.716 (dressing per cent) − 36.82.

These score cards and prediction equations show vividly the lesser importance of dressing per cent as an indicator of fatness under uniform conditions, and its greater importance in comparing results obtained in different places or in different years or with different classes of animals.

One very evident reason why dressing per cent and per cent of offal fat combine to make a better indicator of fatness than either does alone is that, except in so far as they both indicate fatness, they are mutually antagonistic. That is, a high percentage of offal fat would mean a low dressing per cent if all other conditions were equal. This is apparent from the following formula:

$$\text{Dressing per cent} = \frac{A}{A+B+C} \quad \text{and per cent of offal fat} = \frac{B}{A+B+C}$$

where

A = weight of dressed carcass,
B = weight of offal fat,
C = weight of noncarcass parts other than offal fat.

An increase in B unaccompanied by a proportionate increase in A and C will mean a lower dressing per cent and a higher per cent of offal fat. Likewise, an increase in A unaccompanied by proportionate increase in B and C will mean a higher dressing per cent and a lower per cent of offal fat. Knowing this relation one would expect to find a negative correlation between dressing per cent and per cent of offal fat, unless there is some common cause which makes A vary somewhat in proportion to B. That fatness is this common cause is shown by the fact that the partial correlation coefficient between dressing per cent and per cent of offal fat (for a constant per cent of fat in the entire animal) is negative, being −0.33 when only the 30 beef steers from Missouri are considered and −0.46 when all 43 animals are included.

Before leaving this subject it may be well to consider the limits of accuracy of estimations of fatness made by using these equations. For the entire 43 head the standard deviation of per cent of fat in the entire body is 9.08 per cent and the probable error for any one animal at random is 6.12 per cent. The use of the prediction equation will reduce the standard error of estimate to only 35.7 per cent of the original standard deviation (as shown by the multiple correlation coefficient of +0.934). The probable error of the estimated value of the fatness of a single animal will be 35.7 per cent of 6.12 per cent,

or 2.18 per cent. For groups of cattle the probable error of the estimated fatness will vary inversely as the square root of the number of animals in the group. This means that the probable error of the estimated value would equal the above figure (2.18 per cent), divided by the square root of the number of animals considered. Thus, for the ordinary experimental lot of 10 animals the probable error of the estimated percentage of fat in the entire live animals would be 0.69 per cent. For a group of steers of similar breeding slaughtered under the same conditions (using the data for the 30 beef-bred steers from Missouri) the standard deviation is 9.93 per cent and the probable error for a single individual is 6.70 per cent. The use of the prediction equation will reduce the standard error of estimate to 25.9 per cent as large as the original standard deviation (as shown by the multiple correlation coefficient of $+0.966$), and therefore the probable error of the estimated fatness of a single animal will be 1.74 per cent and the probable error for the average of a group of 10 will be 0.55 per cent. It will be seen from the size of these probable errors that carrying the figures for percentage of fat in the entire animal to the second decimal place is a useless procedure, and that many of the figures given in this paper are carried out to more decimal places than are significant. They are left in this extended form, however, in order to show the derivation of the equations and to make it easier to add any future data which may be obtained. It is the writer's suggestion that the prediction equations should be used as given and that the figure thus obtained for percentage of fat in the entire animal should be used only to the first decimal place.

USE OF A COMBINATION OF DRESSING PER CENT AND PER CENT OF CAUL FAT TO PREDICT THE PER CENT OF FAT IN AN INDIVIDUAL ANIMAL

It is sometimes desirable to study the fatness of individual animals within a lot. For this purpose dressing per cent (based on the feed-lot weights and warm-dressed weights) is very easily obtained. Also, it is not much trouble to weigh the caul fat for each steer separately (if the investigator will get the weights himself), and without a very great deal of trouble it is possible to weigh the ruffle fat individually. It is hardly practicable to go beyond that with individual weights of offal fat.

The weights of the caul fat were given for most of the animals included in this study, but the ruffle was not given separately. If the opinions expressed by the practical packing men are correct, that the proportion of caul to ruffle varies widely in different classes of cattle, it is probably unsafe to use the per cent of caul for estimating fatness except between animals which are of the same breed, age, and sex. Even under such conditions it is likely that it is not as accurate an indicator as the combined weights of caul and ruffle. However, the writer has no figures on the ruffle fat for these 43 animals, and so can not compare the accuracy of caul and ruffle combined with the accuracy of caul alone. It may be seen, however, how much the accuracy of prediction for an individual animal is increased when both the dressing per cent and the per cent of caul fat are considered as compared to its accuracy when based upon either one alone.

The correlation between dressing per cent and per cent of caul fat is $+0.570 \pm 0.077$ for the entire 35 animals for which caul weights

were given. For the 26 beef-bred Missouri steers this correlation was $+0.745 \pm 0.059$. Using the former figures, the coefficient of multiple correlation is $+0.917$, which is a decidedly higher correlation than the $+0.775$ between fatness and dressing per cent, or the $+0.845$ between fatness and per cent of caul to live weight. The prediction equation (multiple regression equation) is as follows:

Per cent of fat in live animal $= 0.861$ (dressing per cent) $+ 7.684$ (percentage of caul to live weight) $- 40.24$ per cent.

The standard error of estimate is about 39.9 per cent as large as the original standard deviation, thus making the probable error of the estimated fatness for a single animal about 2.60 per cent.

Using only the figures for the 26 Missouri steers (which are probably more applicable to most cases on account of the uniformity of the conditions under which they were taken), the coefficient of multiple correlation is $+0.941$, which is somewhat of an improvement over the $+0.865$ between fatness and dressing per cent and the $+0.892$ between fatness and per cent of caul fat to live weight for these same 26 steers. The prediction equation is as follows:

Per cent of fat in live animal $= 0.936$ (dressing per cent) $+ 9.073$ (percentage of caul to live weight) $- 44.96$ per cent.

The standard error of estimate is only about 33.8 per cent as large as the original standard deviation, thus making the probable error of the estimated fatness for a single animal about 2.33 per cent.

It is the writer's opinion that the latter equation is the more accurate for use in comparing steers of uniform breeding. Where different sexes or ages or breeds are to be compared it probably will be best to use total offal fat, and to use caution in drawing conclusions even from that. If individual comparisons are to be made between heifers within a lot the latter equation, involving caul fat and dressing per cent, will probably furnish a good relative guide to their fatness, but it may give absolute figures which are too high (on account of the larger proportion of caul fat which heifers are popularly supposed to have) to be used in comparison with a group of steers. A difference in age may also make the absolute figures wrong, although it must be admitted that the data on which this article is based showed no clear evidence of any such effect of age independent of the changes in fatness which normally take place with age.

PER CENT OF BONE IN THE DRESSED CARCASS

In many experiments, especially those comparing animals of different types or ages and those in which minerals are fed, it would be helpful in interpreting the results to know what proportion of bone was in the sides of dressed beef. A chemical analysis is not necessary to determine the percentage of bone to meat but it is necessary to determine the fatness of the meat, and Dechambre (*2*) and Mackenzie and Marshall (*10*) have shown that fatness has a great deal of influence on the percentage of bone in meat.

The most practical indicator of the percentage of bone in the dressed sides seemed likely to be the weight of the four legs, below the knees and the hocks, as removed by the butchers in the packing plants. This weight includes all of the legs below the knee and hock joints, except the skin and the dewclaws, which are removed before the legs are unjointed. This weight can be obtained without

much trouble in any packing plant. The correlation between the percentage of bone in the dressed sides and the percentage which the legs are of the live animal (as shown in fig. 7) is $+0.898 \pm 0.020$ for the entire 43 head for which the data were obtained, and is $+0.948 \pm 0.012$ for the 30 beef steers from Missouri These are both high correlations, the standard error of estimate being 44.0 per cent and 31.8 per cent (respectively) as large as the original standard deviations. It is evident, however, that both variables are influenced to a marked extent by fatness, because increases in fatness would lower both of the bone percentages more or less together and decreases in the amount of fat would raise both the bone percentages more or less together. Since the data cover a wide range of fatness, this effect of variations in fatness may account for much of the correla-

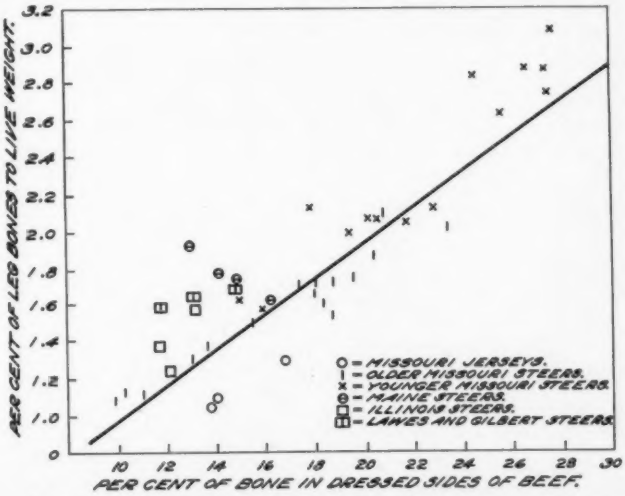


FIG. 7.—The relation between percentage of leg bones to live weight and percentage of bone in dressed sides of beef. The straight line is the equation $x=10.22y+0.04$, and is based on only the 24 Missouri steers which do not show the abnormally high percentage of leg bones to live weight

tion between the percentage of bone in the dressed carcass and the percentage of leg bones to live weight.

Figures 8 to 9 show the relation of fatness to percentage of bone in the dressed carcass and to the percentage of leg bones to live weight. Both of these relations are slightly nonlinear, due especially to the six young, thin Missouri steers which have an unusually high percentage of bone and of legs but a very low percentage of fat. That this is an effect of age seems fairly certain from the fact that all three of the calves slaughtered at 3 months of age are in this group of six which have such an extremely high proportion of leg bones. The other three are the two most poorly nourished of the three which were slaughtered when a little older than 5 months, and the most poorly nourished one of the three which were slaughtered at a little over 8 months of age.

Due to the nonlinearity of the relations shown in Figures 8 and 9 the use of the coefficient of correlation is not completely justified, but on account of the influence of age as well as fatness it was not thought worth while to work out an "index of correlation," and instead four coefficients of correlation were worked out for each of Figures 7, 8, and 9. One included all individuals, one included all individuals except the six young Missouri steers which showed such high percentages of bone, one included all Missouri steers, and the fourth included only the 24 Missouri steers which did not show the unusually high percentages of bone. The last probably most nearly shows conditions as they would exist in an ordinary feeding trial, but even it is not completely free from the influences of age (Table II).

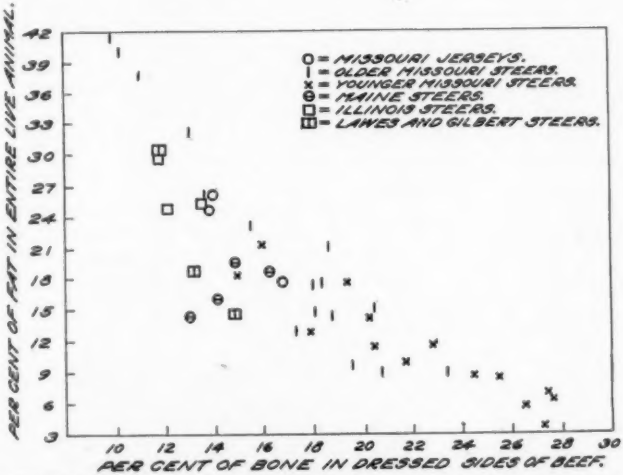


FIG. 8.—The relation between percentage of fat in entire live animal and percentage of bone in dressed sides of beef

TABLE II.—*Correlations, and also the partial correlation coefficients, between percentage of bone in dressed sides and percentage of leg bones to live weight (fatness being constant)*

Group	rBone . Leg bone	rBone . Fatness	rFatness . Leg bones	Fatness rBone. Leg bones
43 head	+0.898±0.020	−0.860±0.027	−0.827±0.020	+0.651
43 less 6	+.774± .044	−.834± .034	−.833± .034	+.260
30 steers	+.948± .012	−.910± .021	−.843± .030	+.811
30 less 6	+.914± .023	−.927± .019	−.906± .025	+.467

The exclusion of the six young, thin steers slightly lowers the primary correlation coefficient between per cent of bone in dressed sides and per cent of leg bones to live weight. The exclusion of these six steers markedly lowers the partial correlation coefficient between these same two variables, with fatness constant. The lower values of the partial coefficients show that much of the primary correlation was due to fatness. The effect of excluding the six young, thin steers shows

that much of the primary correlation coefficient between percentage of bone in the dressed sides and percentage of leg bones to live weight was due to the changes of growth. That is, in the very young, thin steers the skeleton is at a more advanced stage of development than the muscles or fat. Probably some influences of age and growth still appear, even in the smallest of the partial correlation coefficients.

It seems safe to conclude that the weight of the leg bones is a good indicator of the proportion of bone in the dressed sides of beef, but that this is largely a secondary effect of the primary factors of age and fatness. If animals uniform in age and fatness are being compared the indicator will not be nearly so accurate, but there will still be a significant positive correlation between percentage of bone in the dressed sides and percentage of leg bones to live weight. Whether breed and sex differences would affect the accuracy of the indicator is not known.

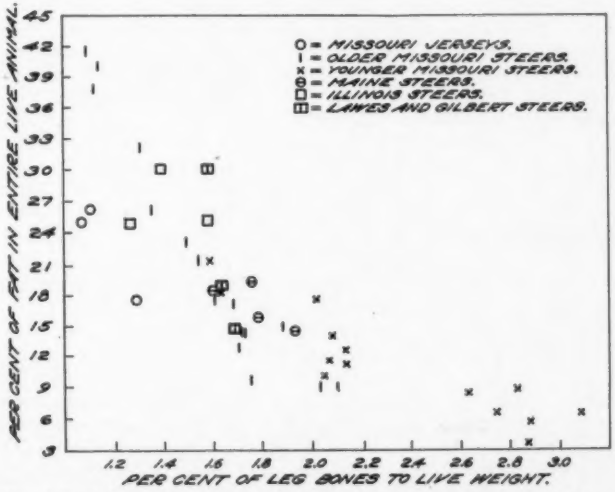


FIG. 9.—The relation between percentage of fat in entire live animal and percentage of leg bones to live weight

Probably the most reliable prediction equation would be that based upon the 24 Missouri steers, since steers as thin as the other 6 Missouri steers are not often slaughtered commercially. That equation is as follows:

> Per cent of bone in dressed sides $= 0.04 + 10.22$ (percentage of leg bones to live weight).

The probable error of the estimated value for a single individual would be about 1.01 per cent. On account of the wide range in the fatness of the animals from which this equation was derived caution should be used in applying it to other cattle. Figure 7 shows the line representing this equation, and it will be seen that the line does not fit equally well the younger and the older steers. The steers under 1 year of age show more variation and would seem to be better fitted by a line with a steeper slope. The steers over 1 year of age show very little variation from a straight line which would have less

slope than the one shown. The numbers of the steers are probably too small to justify more detailed calculations in regard to this point, but it should be kept in mind that the use of this equation is apt to result in values for the percentage of bone in dressed beef which will be slightly too high for very fat mature steers and too low for thin mature steers.

RELATION OF DRESSING PER CENT TO PER CENT OF BONE

On the basis of general observations it is rather commonly believed by animal husbandmen that cattle with small bones have a higher dressing per cent than big-boned cattle, other things being equal. The relations between dressing percentage and percentage of bone are slightly curvilinear, and therefore the cattle were studied separately in the same four groups used previously in studying the relation between percentage of bone in the dressed meat and the percentage of leg bones to live weight. It is obvious that the degree of fatness will have a great deal of influence upon both of these variables, and therefore that it will be necessary to calculate a partial coefficient of correlation between dressing per cent and per cent of bone in dressed meat, fatness being constant. The necessary correlation coefficients are shown in Table III. From this table it will be seen that the very real tendency for a high dressing per cent to be associated with a low percentage of bone is very largely a result of the effect of fatness upon both of these variables. The partial correlation coefficients show a general tendency to be negative, but are so small that their significance is doubtful. It seems safe to conclude that, aside from their relations with fatness, dressing per cent and per cent of bone have little or no relation to each other. This, of course, does not mean that size of bone may not have a causal relation to fatness, and through that relation to dressing per cent. Thus it might be that fine-boned cattle tend to get fatter than large-boned cattle under the same conditions. The data in this paper tell nothing about that. Also, it is possible that size of bone as measured by cannon circumference may not be perfectly correlated with the weight of the bones in the dressed meat. If it is not, then size of bone studied from the standpoint of cannon circumference might show quite different relations with dressing per cent.

TABLE III.—*Primary correlation coefficients between dressing per cent, percentage of bone in dressed meat, and percentage of fat in entire live animal. Partial correlation coefficients between dressing per cent and percentage of bone in dressed meat, fatness being constant*

Group	r Bone . Dressing	r Bone . Fat	r Fat . Dressing	Fatness r Bone . Dressing
43 head	−0.706±0.052	−0.860±0.027	+0.760±0.043	−0.158
43 less 6	− .686± .059	− .834± .034	+ .722± .053	− .220
30 steers	− .766± .051	− .910± .021	+ .842± .036	+ .001
30 less 6	− .778± .054	− .927± .019	+ .828± .043	− .050

The whole problem of size of bone in its relation to meat value, to the desirability of cattle as feeder cattle, and to the desirability of cattle for range-beef production is a large one and is largely outside the scope of this paper. Perhaps the facts just presented may make some contribution toward its solution.

USE OF FATNESS INDICATORS IN THE INTERPRETATION OF FEEDING EXPERIMENTS

In Part I of Bulletin No. 309 of the Texas Agricultural Experiment Station (7) are given the results of a feeding experiment designed to test out the desirability of adding corn to a ration of cottonseed meal and hulls for fattening steers. As was expected, the lot receiving the corn made greater gains, but more expensive gains, than those which received only meal and hulls, and the question of profit resolved itself very largely into a question of the relative desirability of the two lots from the butcher's viewpoint after the feeding was completed, and the question of efficiency of the two rations for producing gain in weight depended for its answer very largely upon what proportion of the gain made by each lot was fat and what part was protein or material other than fat. In that bulletin is this statement:

> * * * it will be seen that Lot III dressed out 1.14 per cent heavier than Lot IV and possessed over 50 per cent more internal fat than Lot IV. Among partially fat animals of the same breeding and the same treatment previous to fattening the amount of this internal fat may be taken as a fair indication of the total amount of fat which they carry. This difference in the amount of internal fat agrees fairly well with the difference in total gain which they made in the feed lots at Spur, in which Lot III gained about 40 per cent more total weight than Lot IV.

It would have been more satisfactory to have had a more definite knowledge of the amount of fat and the amount of nonfat gained by each lot. An estimate of this can be obtained by using the prediction equation derived from the data for the 30 beef-bred steers from Missouri (since Lots III and IV in Texas bulletin 309 were quite uniform, being almost all half brothers or more closely related and all coming from the same herd). The data are as follows:

On basis of final feed-lot weights	Lot III	Lot IV
Warm dressing per cent	56.31	54.85
Per cent of offal fat	5.93	3.91

From the equation the percentage of fat in the entire live animals is 31.07 for Lot III and 20.63 for Lot IV, and the deduction can be made that the average steer in Lot III contained in his body 149 pounds more of true fat than did the average steer in Lot IV. If the equation derived from data from all the 43 head discussed in this paper is used the percentages of fat in the live animals are 25.02 and 17.31, and the average Lot III animal contains in its body 112 pounds more fat than the average Lot IV animal. The use of the equation involving only dressing per cent gives values of 13.94 per cent and 11.34 per cent, which are certainly much too low, while the use of the equation involving only offal fat gives values of 37.68 per cent and 24.95 per cent, which probably are too high. The disagreement among the results of the four equations is due to the varying amount of emphasis placed upon dressing per cent by the different equations. As already noted, there is an indication in the dressing per cent figures that the slope of this equation would be much steeper if it were confined to older steers. If that is true it explains why the use of the dressing per cent equation gives too low values in the case of these steers, which were almost mature. It is also probable that the very bulky nature of their ration caused their

final feed-lot weights to include a rather unusual amount of "fill," which would account for the low dressing per cent. All four equations agree that the Lot III steers were fatter than the Lot IV steers, although the estimates of the additional amount of fat in the average Lot III steer vary from 43 to 181 pounds.

Because of the greater uncertainties about the figures based upon dressing per cent, the writer is inclined to place most confidence in the results obtained by the method which values dressing per cent the least without neglecting it altogether. That is the combination equation based upon the 30 Missouri steers. Moreover, the results of this equation are in closest agreement with the judgment of the practical experts as to the condition of the animals if the figures given by Murray (*14*) for the percentage of fat in animals of different grades are accepted.

As shown by the live weights the average steer in Lot III gained 386 pounds and the average steer in Lot IV gained 289 pounds, a difference of 97 pounds. If the estimation of 149 pounds more fat at slaughtering time in the average Lot III steer than in the average Lot IV steer is accepted, and it is assumed that they were equally fat at the beginning of the feeding (as they probably were, approximately, because they were divided into two lots with that as one of the main points on which division was based), then it follows that the average Lot III steer gained 149 pounds more of fat and 52 pounds less of nonfat live weight than the average Lot IV steer. Assuming that the equation used was the one best suited to this case, the probable error of the estimated difference in fat in the gains made by the average steer of the two lots would be a little more than 9 pounds. Deviations of this estimate from the true value may account for part of the extra 52 pounds of increase in nonfat live weight attributed to the Lot IV steers. Part of that 52 pounds may be attributed perhaps to the greater bulkiness of the ration fed to the Lot IV steers, this causing them to develop more paunchiness than the Lot III steers and thus to be weighed in the final feed-lot weighings with more feed and water in their digestive tracts than the Lot III steers had. Part of that 52 pounds may actually represent an increased growth of muscle, or bone, stimulated by the nature of the ration, although there is little evidence from studies of nutrition to support such a possibility. Assuming that these figures of 149 pounds more of fat and 52 pounds less of nonfat live weight are correct, the following calculations would be logical:

If the fat-free live weight contains about 28 per cent dry matter (*12*), and a pound of fat contains about 1.67 times as much energy as a pound of fat-free dry matter, then each pound of increase in fat would have required about six times as much energy from the food as each pound of fat-free increase in live weight. Thus the 52 pounds of extra gain in nonfat live weight made by Lot IV is equivalent, on an energy basis, to about 9 pounds of fat, and the average steer of Lot III can be regarded as having produced 140 pounds more of fat per steer in 120 days on a ration which was calculated to have supplied 807.6 more therms of net energy per steer in that period. Using Armsby's (*1*) figure of 4.309 therms per pound as the energy content of a pound of fat, the 140 pounds of extra fat gained will account for 603.3 therms of the excess fed to Lot III, leaving only 204.3 therms of excess net energy per steer unaccounted for. This is less than 10

per cent of the estimated net energy content of the total ration fed to the Lot III steers. Whether it is to be ascribed to faulty estimation of the amount of fat in the gain, to a higher maintenance requirement of the Lot III steers after they had become fat, to less perfect digestion by them on account of the much larger quantity of grain in their ration, or whether the net energy values used for cottonseed meal or cottonseed hulls, or both, were lower in comparison to the net energy value used for ground shelled corn than they should have been, can not be determined from these data.

If the estimated figures of 149 pounds more fat and 52 pounds less nonfat live weight are regarded as incorrect, and one prefers to regard the difference in gain made by the two lots (97 pounds per head) as composed entirely of fatty tissue of the maximum degree of fatness (about 95 per cent) and to suppose that the remainder of the gains made by the Lot III steers were identical in composition with the entire gains made by the Lot IV steers, then this extra 97 pounds of fatty tissue would account for about 400 therms of the excess net energy calculated to have been fed to Lot III, and about 400 therms would remain unaccounted for in the rations fed to each Lot III steer. This is about 20 per cent of the entire ration fed to the Lot III steers and may be accounted for by some of the reasons enumerated in the preceding paragraph.

The results of these calculations lack much that is to be desired in the way of exactness, but they seem to be an improvement over the statement in Texas bulletin No. 309 (7) that the steers of Lot III required 3.55 therms per pound of gain and the steers of Lot IV required 1.93 therms per pound of gain, and the necessarily general statement that much of this difference must have been due to the larger proportion of fat which probably characterized the gains of Lot III. These calculations give a better picture of what actually took place in these steers during the feeding process, and therefore they seem quite worth while, although they are estimates based upon the average performance of other steers and they can not be considered quite as exact as things which could be weighed or measured on the Lot III and Lot IV steers themselves.

In the other comparison in Texas bulletin No. 309—i. e., the comparison between steers which were not quite 2 years old (Lot I) and steers which were not quite 3 years old (Lot III), both lots being fed the same ration—the calculations can not be carried quite so far. Here it can not be assumed that the two lots contained the same percentage of fat when they went on feed. Not only did the older steers probably contain a larger percentage of fat, because that is usually the case with older cattle, but the younger cattle were also judged to be thinner at the beginning of the feeding. The dressing per cent of Lot I (basis of final feed-lot weights and warm-carcass weights) was 53.99 per cent and the per cent of offal fat was 4.54. Using the equation derived from the 30 beef-bred Missouri steers, the per cent of fat in the average steer of Lot I is 22.95 per cent, which agrees well with the opinions of the men who saw the cattle and also with the prices paid for them. However, this figure can not be used in testing the uses to which the net energy in the rations was put without making some sort of an assumption about the percentage of fat in the Lot I steers when they went on feed. Probably all that is justified is the conclusion that since the older steers were much fatter at the close of the feeding period their gains contained a higher

proportion of fat than did the gains of the younger steers. It is possible that the apparently greater efficiency of the younger cattle may be entirely explained on this basis.

It would appear that if this relation between dressing per cent, offal fat, and fatness is to be used to the greatest advantage, the comparison must be between two lots which can be assumed to be of the same degree of fatness at the beginning, or, if that is not possible, several steers from each lot must be slaughtered as a sample at the beginning of the feeding test.

COMPOSITION OF THE RIB FLESH AS AN INDICATOR OF THE FATNESS OF THE ENTIRE ANIMAL

In experiments where only a part of the beef animal has been analyzed for fatness (*6*, *10*), the portion most frequently used has been the rib cut. In a letter to the writer C. Robert Moulton, who was responsible for collecting and analyzing much of the Missouri data used in this study, says in reference to the composition of the

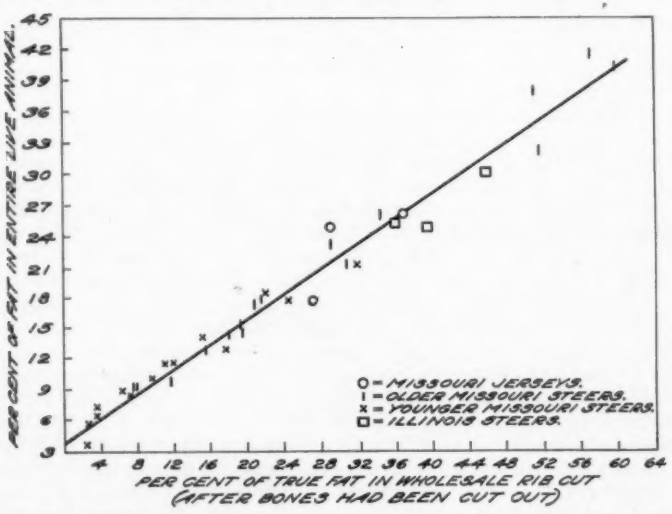


FIG. 10.—The relation between percentage of fat in the entire animal and the percentage of fat in the flesh of the wholesale rib cut. The straight line is the prediction equation, $y=0.603x+3.92$ per cent, and is based on all animals shown

wholesale rib cut: "When at Missouri Doctor Trowbridge and myself from a survey of our data came to the conclusion that the wholesale rib cut rather adequately represented the carcass."

In the rather extensive plans now being made by a number of research agencies for the study of meat composition and quality, the question of analyzing some part of the dressed carcass is frequently considered. Therefore it may be appropriate to consider here the reliability of the fatness of the flesh of the wholesale rib cut as an indicator of the fatness of the entire animal. Of course the chemical analysis of the rib flesh would require the destruction of that cut as well as an expense for the cost of the chemicals and labor used in the analysis. Therefore such procedure would not

ordinarily be practicable with cattle sold to commercial packing plants. However, the experimenter might arrange in advance for the purchase of the wholesale rib cuts from the packing plant for analytical purposes. Certainly many more investigators could do this than could undertake to analyze entire steers.

Figure 10 shows the relation between the percentage of fat in the entire live animal and the percentage of fat in the flesh of the wholesale rib cut. The 30 Missouri steers, the 3 Missouri Jerseys, and the 3 Illinois steers are included. The relation seems to be linear, and the coefficient of correlation is $+0.987 \pm 0.003$.

The standard error of estimate is only 16 per cent as large as the original standard deviation, and the probable error of the estimated fatness for a single steer is about 1.04 per cent. The equation for predicting fatness is:

> Percentage of fat in live animal = 0.603 (percentage of fat in rib flesh) + 3.92 per cent.

This indicator is more reliable than any one of those already discussed or any combination of them. Moreover, differences in sex and breed of the cattle slaughtered and differences in slaughter technique seem to have less influence upon this indicator than upon the others already discussed. It would seem, therefore, that the analysis of the flesh from the wholesale rib cut might be well worth while in experiments in which it is important to determine exactly the degree of fatness of the entire animal. For the worker who has the means of making these analyses the use of the other indicators of fatness would probably be of interest only for checking the results of the analysis of the rib flesh. For the very large number of animal husbandry workers, however, who are not in a position to buy and analyze the ribs of the cattle they have fed, the use of the indicators previously discussed should prove helpful.

SUMMARY

The object of this study was to find whether there were any reliable quantitative indicators of the percentage of fat in the bodies of cattle and of the percentage of bone in the dressed sides of beef which could be obtained easily in commercial packing plants. This study is based upon the published records of the slaughtering of 30 steers and 3 cows at the Missouri station, 4 steers at the Maine station, 3 steers at the Illinois station, and 3 steers slaughtered by Lawes and Gilbert in England.

The percentage of true fat in the lean and fat flesh and the percentage of true fat in the entire live animal were almost perfectly correlated.

The percentage of true fat in offal fat and in kidney fat bears a very definite relation to the fatness of the animal, but that relation is curvilinear and not adopted for use as an indicator.

The percentage of offal fat to live weight was found to be the most reliable single indicator of fatness.

The percentage of caul fat to live weight was found to be the next most reliable, but it would lose much of its reliability if used in comparing different breeds or sexes.

Dressing per cent was found quite reliable where wide ranges of fatness were compared, but not very reliable within narrow limits of fatness.

A combination of dressing per cent and percentage of offal fat to live weight is recommended as the most reliable indicator of fatness for groups of cattle.

A combination of dressing per cent and percentage of caul and ruffle fat to live weight is recommended as the most reliable indicator of fatness for individual animals.

The percentage of leg bones to live weight is shown to be a very good indicator of the percentage of bone in the dressed sides of beef, but this is largely a secondary correlation resulting from extreme differences in fatness and age among the animals studied.

The percentage of bone in dressed meat is negatively correlated with dressing per cent to a very high degree. This correlation is largely a secondary result of the high correlation between the degree of fatness and each of these two percentages. There is little or no evidence of any relation between dressing per cent and percentage of bone in dressed meat when the degree of fatness is constant.

Prediction equations are given for the use of these indicators, and their limits of accuracy are discussed. The most reliable and useful prediction equations found are as follows (final feed-lot weight being used as the live weight in all formulas where live weight is involved):

(*a*) Fatness of groups of cattle:

Per cent of fat in entire live animal = 4.65 (per cent of offal fat to live weight) + 0.716 (dressing per cent) − 36.82 per cent.

(*b*) Fatness of individual animals of the same sex:

Per cent of fat in entire live animal = 9.073 (per cent of caul fat to live weight) + 0.936 (dressing per cent) − 44.96 per cent.

(*c*) Percentage of bone in dressed carcass = 10.22 (percentage of leg bones to live weight) + 0.04 per cent.

The use of such estimations in interpreting feeding experiments is illustrated by an example, using data from Texas bulletin No. 309.

The percentage of fat in the flesh of the wholesale rib cut is a more accurate indicator of the degree of fatness of the entire animal than any of the other indicators studied. Where the chemical analysis necessary for this determination is practicable its use is recommended. Its prediction equation is:

Per cent of fat in entire animal = 0.603 (per cent of fat in rib flesh) + 3.92 per cent.

LITERATURE CITED

(1) Armsby, H. P.
1917. The nutrition of farm animals. 743 p., illus. New York.

(2) Dechambre, P.
1918. Relation entre le poids du squelette et le poids de la viande nette chez les bovins de boucherie. Compt. Rend. Acad. Agr. France 4: 25–28.

(3) Haecker, T. L.
1920. Investigations in beef production. Minn. Agr. Exp. Sta. Bul. 193, 110 p., illus.

(4) Hall, L. D., and Emmett, A. D.
1912. Relative economy, composition and nutritive value of the various cuts of beef. Ill. Agr. Exp. Sta. Bul. 158: 135–233, illus.

(5) HAMMOND, J.
1920. ON THE RELATIVE GROWTH AND DEVELOPMENT OF VARIOUS BREEDS AND CROSSES OF CATTLE. Jour. Agr. Sci. [England] 10: 233–289.
(6) HUNGERFORD, J. D., and FOSTER, L.
1921. THE UTILIZATION OF FEED BY RANGE STEERS OF DIFFERENT AGES. III. ALFALFA HAY AND COTTONSEED MEAL. IV. ALFALFA HAY, MILO MAIZE MEAL, AND COTTONSEED MEAL. N. Mex. Agr. Exp. Sta. Bul. 128, 92 p., illus.
(7) JONES, J. M., LUSH, J. L., and JONES, J. H.
1923. I. FATTENING STEERS ON COTTONSEED MEAL AND HULLS WITH AND WITHOUT CORN. II. THE INFLUENCE OF AGE ON FATTENING STEERS. Tex. Agr. Exp. Sta. Bul. 309, 31 p., illus.
(8) JORDAN, W. H.
1896. THE RELATION OF FOOD TO THE GROWTH AND COMPOSITION OF THE BODIES OF STEERS. Me. Agr. Exp. Sta. Ann. Rpt. 1895 (2): 36–77.
(9) LAWES, J. B., and GILBERT, J. H.
1860. EXPERIMENTAL INQUIRY INTO THE COMPOSITION OF SOME OF THE ANIMALS FED AND SLAUGHTERED AS HUMAN FOOD. Phil. Trans. Roy. Soc. London (1859) 149: 493–680.
(10) MACKENZIE, K. J. J., and MARSHALL, F. H. A.
1918. BEEF PRODUCTION. Jour. Bd. Agr. [London] 25: 623–640.
(11) MOULTON, C. R., TROWBRIDGE, P. F., and HAIGH, L. D.
1922. STUDIES IN ANIMAL NUTRITION. II. CHANGES IN PROPORTIONS OF CARCASS AND OFFAL ON DIFFERENT PLANES OF NUTRITION. Mo. Agr. Exp. Sta. Research Bul. 54, 76 p., illus.
(12) ——— TROWBRIDGE, P. F., and HAIGH, L. D.
1922. STUDIES IN ANIMAL NUTRITION. III. CHANGES IN CHEMICAL COMPOSITION ON DIFFERENT PLANES OF NUTRITION. Mo. Agr. Exp. Sta. Research Bul. 55, 88 p., illus.
(13) ——— TROWBRIDGE, P. F., and HAIGH, L. D.
1923. STUDIES IN ANIMAL NUTRITION. V. CHANGES IN THE COMPOSITION OF THE MATURE DAIRY COW WHILE FATTENING. Mo. Agr. Exp. Sta. Research Bul. 61, 20 p.
(14) MURRAY, J. A.
1919. MEAT PRODUCTION. Jour. Agr. Sci. [England] 9: 174–181, illus.
(15) TROWBRIDGE, P. F., MOULTON, C. R., and HAIGH, L. D.
1919. COMPOSITION OF THE BEEF ANIMAL AND ENERGY COST OF FATTENING. Mo. Agr. Exp. Sta. Research Bul. 30, 106 p., illus.
(16) WALLACE, H. A., and SNEDECOR, G. W.
1925. CORRELATION AND MACHINE CALCULATION. Iowa State Col. Off. Pub., v. 23, no. 35, 47 p., illus.
(17) WRIGHT, S.
1921. CORRELATION AND CAUSATION. Jour. Agr. Research 20: 557–585, illus.

RELATION OF GROWTH IN THE POTATO TUBER TO THE POTATO-SCAB DISEASE [1]

By HURLEY FELLOWS [2]

Associate Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture; formerly Scholar, Department of Plant Pathology, University of Wisconsin

INTRODUCTION

That the common potato scab is a parasitic disease was proved by Thaxter (*17*)[3] in 1891, who called the causal organism *Oospora scabies;* but Güssow (*6*), in 1914, transferred the organism to the genüs Actinomyces. Previous to the time of Thaxter's discovery, growers had held several theories as to the cause of potato scab, and had sought in vain for satisfactory methods of control. Since the demonstration of the parasitic nature of the scab, investigations concerning it have dealt chiefly with control methods, with the effect of environmental factors on the production of the disease, and with the cause of resistance. Other investigations have been made of the physiology and taxonomy of the organism.

The experiments reported in this paper were begun as a result of observations made by the writer at the Wisconsin Agricultural Experiment Station while working on the relation of soil temperature to the amount of scab produced on tubers (*9*). It became evident that there was an intimate relation between the rate of growth of the potato tuber and the amount of scab which developed. The tubers which grew most vigorously and became the largest were most severely affected, whereas the small tubers showed only a slight infection. It was also noted that the original seed piece never developed scab lesions, even when planted in soil which was heavily inoculated with the scab parasite. Further observations of several hundred tubers infected with scab revealed the fact that the disease was more severe at the basal end of the tuber than in the apical region. At first thought this might seem to contradict the previous contention that scab development is dependent on growth, unless the tuber, unlike other stems, increases in size more rapidly at the basal end than in the apical region. There was also the possibility that the basal end might be more susceptible to scab than the apical region, or that the tuber might be more susceptible when small than when larger. Further consideration indicated that the tendency for scab localization might be explained on the basis of a time factor, since all observations showed that the base of the tuber possessed the oldest surface tissue. It was thus possible that the disease had been present on the basal end for a longer time and as a result had spread over a larger area.

[1] Received for publication May 26, 1925; issued March, 1926.

[2] The writer makes grateful acknowledgment to L. R. Jones and H. H. McKinney for suggestions in the course of the investigation herein reported. This work was done during the years 1922 and 1923 in partial fulfillment of the requirement for the degree of doctor of philosphy at the University of Wisconsin.

[3] Reference is made by number (italic) to Literature cited, p. 781.

These considerations suggested that the development of scab might be dependent to a large extent on the growth of the host in somewhat the same manner as that suggested by Keitt (*11*) in connection with the increased susceptibility of the young growing leaves and fruit of the apple to *Venturia inaequalis*. Another case of similar nature has been reported by L. K. Jones (*7*) in connection with the immunity of the mature tissue of the raspberry canes to anthracnose. These observations, together with many other known instances of a similar nature, would seem to justify an attempt to determine whether the same is true in the case of potato scab.

A search through the literature showed that little work has been done on the exact manner in which the potato tuber increases in size. Little has been shown, also, regarding the mode of infection by *Actinomyces scabies* and the relative susceptibility of the different parts of the tuber or of the tuber as a whole during different stages of its development. Accordingly, experiments were undertaken to determine the mode of tuber development with especial regard to the method of enlargement, the formation of stomata, lenticels, epidermis, and cork, and the time of tuber setting. The study of stomata, lenticels, epidermis, and cork were made on various portions of single tubers of various sizes. The mode of infection by the organism was given attention. The histology of the lesions was studied in order to determine whether the organism or its products come into direct contact with the more actively dividing cells of the external parts of the tuber and if so, the effect of such contact on these developing tissues.

SOME PHASES OF TUBER GROWTH

METHOD OF STUDY

The studies made of growth processes in the potato tuber were conducted in the field at Madison, Wis., during the summers of 1921 and 1922. These experiments were not designed with a view to finding out all the phases of tuber development from the beginning until growth entirely ceased, but were intended primarily to demonstrate the method of enlargement of tubers from about the time they were one-tenth mature until growth was over. Cytological studies were restricted to those portions of the tuber in which scab might be produced—that is, to the epidermal and corky coverings and the cells immediately beneath. Since tubers vary in shape according to variety, two types were selected which represented fair extremes of length, namely, the Irish Cobbler, which is round, and the Early Ohio, which is elongated (fig. 1). When the tubers were 1 or 2 inches in diameter, the soil was carefully removed from about them and each was marked with India-ink lines. These lines or bands were about 5 millimeters in width and were drawn from the base to the apical end of each tuber. A short line of equal width was made at the growing point in such a way as to intersect the long line at right angles, thus making a cross at the apical end, as shown in Figure 1, C and D. The soil was then replaced, and after varying periods, extending from four to seven days, the soil was again removed and observations made, the spread of the ink band being an index to the amount of growth (pl. 1). Direct measurements were also made in many cases.

In addition to the above-mentioned procedure, a microscopic examination of the marked and unmarked tubers was made to note the location of stomata and lenticels and to observe the method of formation of the latter. Further observations for the same purpose were made on tubers grown in the greenhouse. A microscopic examination was also made of the protective corky and epidermal layers to determine if any difference existed in these layers in different portions of the tuber.

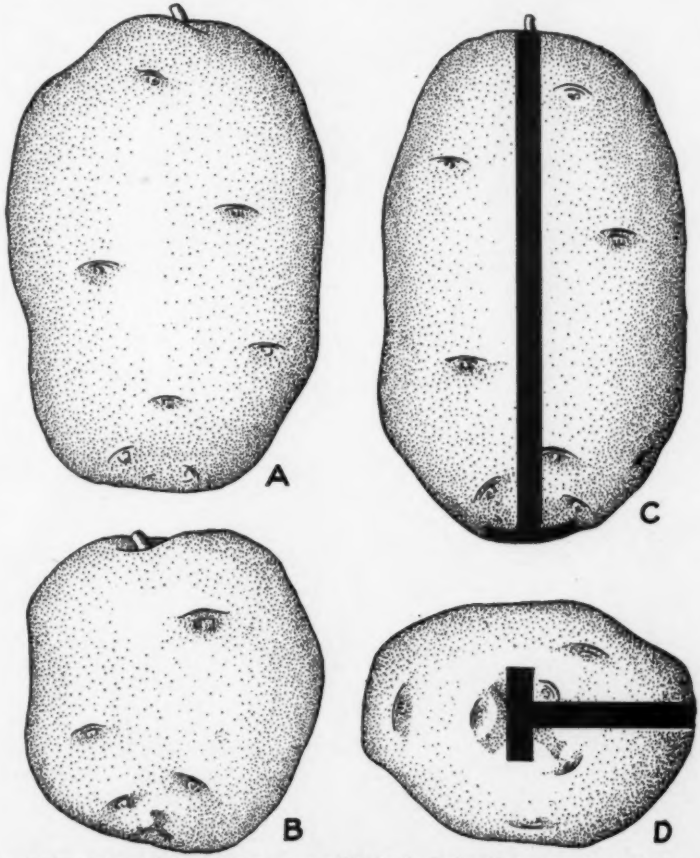
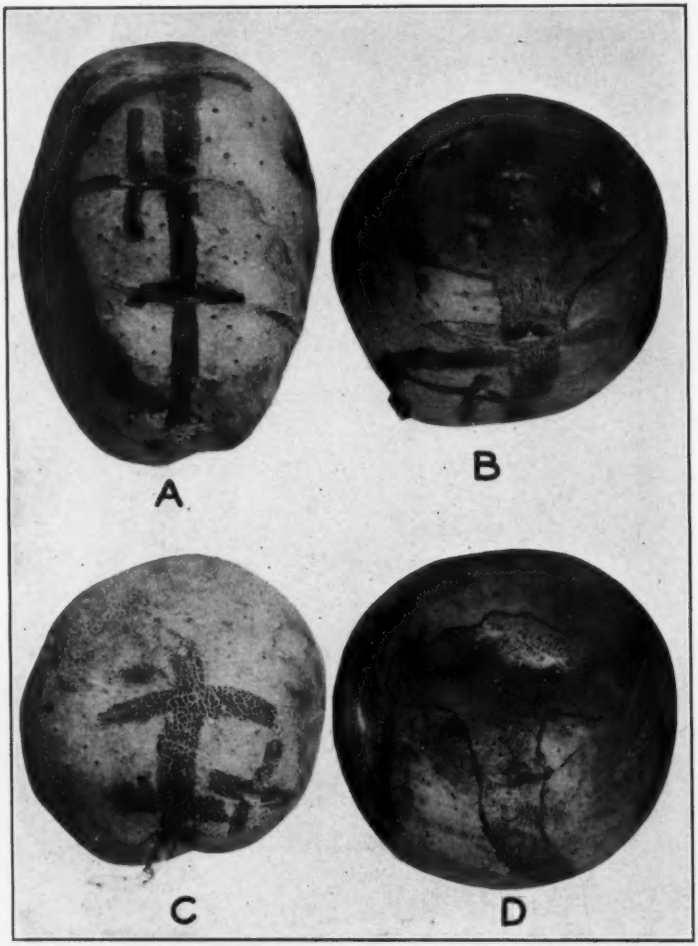


FIG. 1.—A, an elongated type of tuber as exemplified by the Early Ohio variety. B, rounded type of tuber exemplified by the Irish Cobbler variety. C and D, side view of a tuber showing the method of marking growing tubers with India ink. The relative spread of this ink indicates where growth occurs. Plate I shows photographs of certain tubers so marked

RESULTS

It is well to describe the initial stages in tuber formation before discussing the results obtained in connection with the growth studies on tubers of 1 or 2 inches in diameter. Tubers are formed on stolons which are located at the axils of scalelike leaves on underground stems. The first indication of a tuber is a localized swelling of the



Increase in size occurring on various portions of growing tubers as indicated by the diffusion of India ink placed on their surface. The method of marking is illustrated in Figure 1

A.—The ink has not spread on the basal end of a growing elongated tuber, but it has spread on the apical end

B and D.—The apical end of tubers which have grown, following the application of ink. Before growth the ink marks formed a small cross at the apical end; here it is scarcely visible to the eye owing to spread by growth. The apical bud is located near the center of the ink. In order to show more clearly the margins of the original ink marks these were retraced before photographing

C.—Ink has spread on the basal end of the growing round tuber

stolon, usually at or near the tip. At first this swelling is clavate but soon it becomes rounded and later takes the shape characteristic of the potato variety. At the time when the marks were made on the tubers for growth observation, they had had their characteristic shape for some time.

Enlargement

External enlargement takes place in two general directions as in any growing dicotyledonous stem. There is a general increase in length at the apical end accompanied by an increase in diameter. The criterion for this was the relative degree of ink spread and the direct measurements. The manner of growth for all tubers was not uniform, but was modified by the varietal shape of the tuber. Round tubers increased in size rather uniformly in all diameters, as is indicated by the uniform spread of the ink marks over the entire surface, while elongated forms, on the other hand, grew much more rapidly in length (fig. 2), this growth being much more conspicuous toward the apical end (pl. 1, A and B). In no case where measurements were made did a tuber of any variety grow more rapidly in diameter than it did in length (Table I).

Table I.—*Growth of potato tubers in length and width after varying lengths of time*

Days of growth	Tuber No.	Growth in—		Days of growth	Tuber No.	Growth in—	
		Length	Width			Length	Width
		Mm.	*Mm.*			*Mm.*	*Mm.*
4	1	10	10	7	7	16	11
4	2	18	12	7	8	14	6
	Average	14	11	7	9	18	4
				7	10	10	2
				7	11	11	3
6	3	7	2	7	12	12	4
6	4	11	3	7	13	15	7
	Average	9	2.5	7	14	12	11
				7	15	14	10
7	5	18	12		Average	14	7.25
7	6	14	10				

According to Jürgens (*10*), the form of tubers is governed by the relation of the length growth to thickness growth. Artschwager (*1*) found in his researches on the anatomy of the potato plant that the cells which divide and grow to cause the enlargement of a tuber are not primarily those of the cambium. The first swelling of the stolon is caused by an excessive cell division of the pith up to the time tubers are 5 to 10 millimeters in average diameter. From this time on, the cells of the premedullary zone and, to a lesser extent, those of the cortex are responsible for the increase in size. Reed (*16*) maintains that the pith and premedullary zone contribute equally to the tuber enlargement.

Formation of Cork and Epidermis

The most interesting thing from the standpoint of the scab disease is the changes which occur at the apical end as the tuber grows. The new tissue which is being formed there is always covered by a single layer of epidermis during the stage of rapid tuber development. A short time after the epidermis is formed, depending on the speed

of growth, periclinal walls are formed in its cells and in the subepidermal parenchyma cells. These cells, after several such divisions, are transformed into the corky layer by the suberization of their cell walls and by a gradual loss of their contents. The parenchyma cells lying immediately below the corky cells become the phellogen and supply any additions to the original corky layer.

Lutman (*14*), in connection with his studies on immunity of tubers to scab, made a number of tests to determine the thickness of the corky layer in many varieties of potatoes, but he has made no mention of the nature of the protective covering at the apical end. The result of his work, although suggestive of the fact that the thickness of cork has some bearing on the resistance of tubers to scab, offers no clear-cut evidence in support of such a theory.

As maturity approaches, the tuber continues to increase in size, but less rapidly. At this time the entire, or nearly the entire, surface is covered by a corky layer several cells in thickness. There seems to be a somewhat direct correlation between the rate of

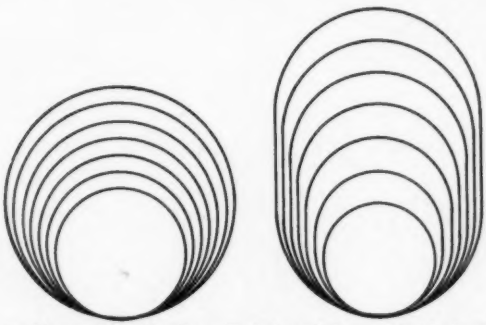
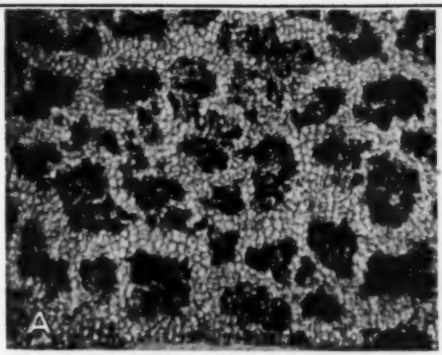


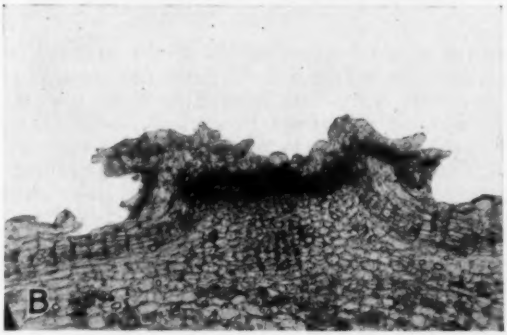
FIG. 2.—Diagram illustrating the method of growth in round and in elongated tubers

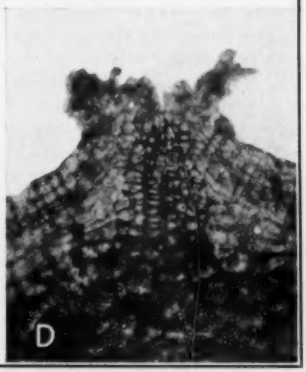
growth and the quantity of epidermis at the apical end; the faster the growth the greater the area of epidermis, and vice versa.

FORMATION OF STOMATA AND LENTICELS

In the new epidermis formed at the apical end stomata are found in abundance. Shortly before the new corky layer appears these become somewhat raised on nipplelike protuberances. Judging by the number of increased cells found in the region below the stomata as compared with the number of surrounding subepidermal cells, one is led to believe that the swelling may be caused by a rapid division in this underlying portion, although the writer has not observed division. Below the stoma, between the cells of the swelling, are found large intercellular spaces which appear darkened in fresh unstained sections (pl. 2, A). This swelling continues, and more intercellular spaces are formed until finally the epidermis or the new corky layer, which sometimes begins to appear by this time, is ruptured and a lenticel is formed. This rupture exposes to the surface the free parenchyma cells, with their large intercellular spaces, at the center of the lenticel. The rapidly dividing cells of the subepidermal layer, which are forming cork at this time, are







A.—The first stage of lenticel formation, a swollen protuberance with a stoma on top and intercellular spaces below. The corky epidermal layer has not yet formed

B.—A lenticel recently broken open. The dividing cells of the subepidermal layer are freely exposed to the surface. The intercellular spaces also extend to the surface

C.—An old lenticel after the corky layer of the tuber has been completed. The cells in the darkened portion of the lenticel crater are heavily suberized

D.—An enlarged portion of the corky covering of a tuber which had been earlier marked with india ink, this ink appearing as darkened areas with the new cells of the corky layer showing between

likewise exposed around the ragged external margin of the lenticel (pl. 2, B). If one cuts and examines older lenticels there seems to be a protective layer or pad of empty cells just outside of and in contact with the parenchyma cells below (pl. 2, C). These parenchyma cells having the intercellular spaces are no longer exposed to the surface; this is also true of the phellogen. The cells of the subepidermal layer which were dividing when the lenticel was first formed, have now become cork and are no longer capable of division. Sections of old, completely formed lenticels when treated with Sudan III show this padlike portion within the crater of the lenticel to be of a fatlike nature. This region gives a reddish-orange reaction, as does also the corky layer covering the surface of the tuber. This reaction indicates that the protective layer over the lenticel is suberized in a manner similar to the corky layer covering the rest of the tuber. Jürgens (*10*) claims that all cells of mature lenticels are suberized.

TIME OF TUBER SETTING AND RELATIVE GROWTH OF INDIVIDUAL TUBERS

In the course of some experiments in the greenhouse it became necessary to examine entire hills of tubers at various periods from the time the tubers were first formed to their maturity. Counts made of the number of tubers present at these different periods indicated that all of the tubers were initiated at approximately the same time. These preliminary observations led to further examination to determine the time of tuber setting and the relative growth of individual tubers. Accordingly, 90 pots of Irish Cobbler potatoes were planted and kept in the greenhouse during the spring of 1923. Several of these were emptied at various intervals and observations made on the number, size, and total weight of the tubers on each plant.

Comparisons as to number and relative weight of tubers, must be made between plants which have an equal number of stems. The removal of the extra stems in some pots, in order to make the number of those remaining equal to those in other pots, is not a successful method, for a part of the severed stem often remains below ground, where it continues to live and produce tubers. These extra tubers introduce an error, yet they can not be disregarded, since their development tends to reduce the size of the tubers on the unsevered stems. The difficulties were met by selecting, from a large population of plants those having an equal number of stems.

TABLE II.—*Results obtained from periodic removals of potato hills*

[Potatoes were all planted at the same time]

Days of growth	Number of stems	Number of tubers having an average diameter in millimeters of—															Total number of tubers	Number of hills	Average number of tubers per hill	Average weight of tubers per hill
		1	2.5	5	7.5	10	15	20	25	30	35	40	45	50	55	60				
																				Gram
55	3	22	25	15	18	12	12	4	1								109	5	21.80	14.3
70	3	7	79	77	28	12	2	0	8	3	6	1	2	1			226	9	25.10	69.4
94	3	15	57	36	27	4	7	4	2	2	1	3	2	0	2	3	165	7	23.57	131.0
43	2	25	23	29	13	7	2	1									100	6	16.60	3.0
70	2	8	39	51	14	11	5	4	2	1	0	3	4	0	1	0	143	8	17.87	72.1
94	2	3	22	31	23	12	4	1	1	1	0	0	3	3	3	1	108	7	15.42	139.1

Out of a population of 90 plants, 21 produced three stems and 21 produced two stems. Of the three-stemmed individuals, 5 hills were dug 55 days, 9 hills 70 days, and 7 hills 94 days after planting; of the two-stemmed individuals, 6 hills were dug 43 days, 8 hills 70 days, and 7 hills 94 days after planting. After each digging observations were made on the average diameter of the tubers, the total number of tubers per hill, and the weight of tubers per hill (figs. 3 and 4; Table II).

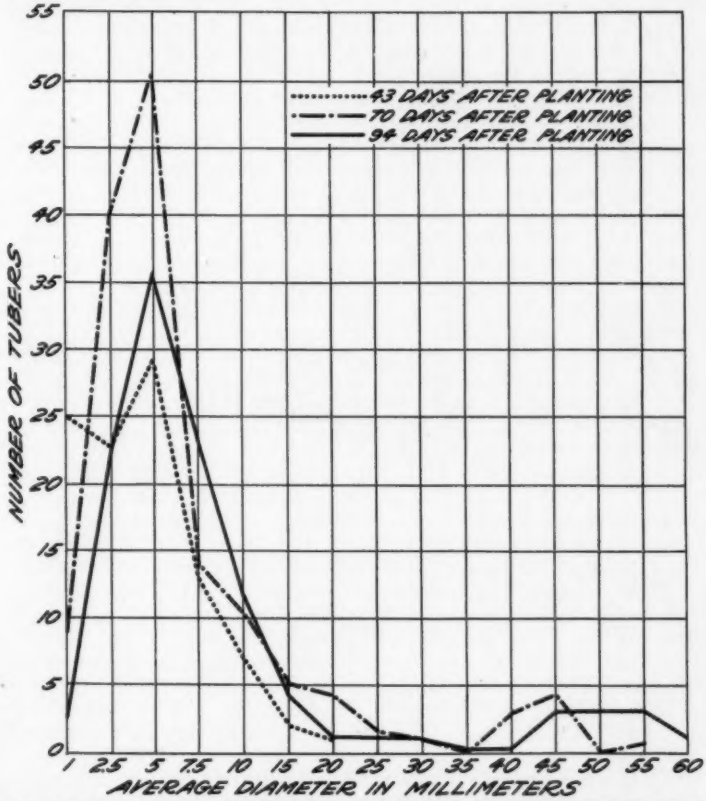


FIG. 3.—Graph showing the number of tubers of various diameters, following three periodic removals of whole hills having two stems each. The majority of the tubers have remained 5 millimeters in diameter, which shows that the smaller tubers have not grown. The larger tubers have increased in size with each consecutive digging

Among the three-stemmed plants, those dug after 55 days showed an average of 21.8 tubers per hill, after 70 days 25.1, and after 94 days 23.4. These figures show an increase for a time in the number of tubers per hill, followed by a decrease. Of the two-stemmed plants, those dug at 43 days after planting showed an average of 16.6 tubers per hill, after 70 days 17.3, and after 94 days 15.4. Thus a slight increase is shown at first, followed by a decrease at the last digging. The last digging of the three-stemmed plants showed a

slightly larger average per hill than the first, but less than the second, while the last digging of the two-stemmed plants showed a decrease in the average number of tubers per hill from the first and second removals. There was a marked increase in weight all through each series (Table II).

The observations noted above, together with the additional data presented in Table II, indicate plainly that all tubers are set at approximately the same time. The average weight of tubers per hill further indicates that growth did occur. The natural question arises: Which tubers grew? Figures 3 and 4, which were made from data on the average size of tubers as coordinated with the number of tubers, throws some light on this subject.

If one considers the plants which had three stems each, it will be seen that at whatever stage of maturity the tubers were dug, the greater number of tubers are 2.5 millimeters in diameter (fig. 4). Now, if the smaller tubers grew appreciably, one would expect these to shift in the graph from the 2.5 millimeters ordinate to one higher; but this does not occur in any of the three series of diggings. The same general principle holds with respect to the plants having two stems each, except that in this case the maximum number of tubers at each consecutive digging had an average diameter of 5 millimeters (fig. 3). This should indicate then that the smaller tubers never grow. Table II shows that the increase in weight was due to the growth of the larger tubers. Those plants dug 55 days after planting each had 12 tubers with a diameter of 15 millimeters, four with a diameter of 20 millimeters, and one with a diameter of 25 millimeters. On the other hand, Table II also shows that those plants which were dug 94 days after planting had one tuber 35 millimeters in diameter, three 40 millimeters, two 45 millimeters, two 55 millimeters, and three 60 millimeters. This increase in size of the larger tubers also holds true for the two-stemmed individuals (Table II). Evidently, then, the increase in weight is due to the growth of the larger tubers and not to the growth of the small ones, which remain nearly stationary in size. If hills of tubers are dug at a time when maturity is approaching, many of the smaller tubers will be found shrunken and disintegrating. This indicates further that some of the tubers which set never grow to any appreciable size, because they are sacrificed to their rapidly growing neighbors, perhaps by resorption of their contents, since they generally shrink without decaying. The relation of this fact to disease will be discussed later.

Clark (*4*) in his work concludes that the difference in size of tubers in individual hills is to be attributed largely to unequal rate of growth rather than to differences in age of the tubers. His observations are based on fairly large populations grown in the field, and it seems reasonable to believe that his conclusions are correct. Clark further states that there was a slight increase in the number of tubers for a time in the early life of the plant, followed later by a decrease. He attributes this decrease to the shrinkage and disintegration of the smaller tubers; the same conclusion that has been reached in the present work. The evidence seems to indicate that all tubers are set at relatively the same time and that the decrease in number is caused by disintegration during the life history of the plant.

It is not the writer's purpose to discuss at length dominance and inhibition (*3, 12, 15*) from a physiological viewpoint, but simply to mention them and their possible application to the case of growing and nongrowing tubers. In axiate plants, the fact has long been known that a physiologically active growing portion may inhibit the growth of other parts at a more or less remote distance. This has been found true in the case of growing tips, roots, leaves, and other portions of the plant. The removal of these active parts has been shown to allow the dormant parts to resume activity. Various hypotheses have been advanced to account for this phenomenon,

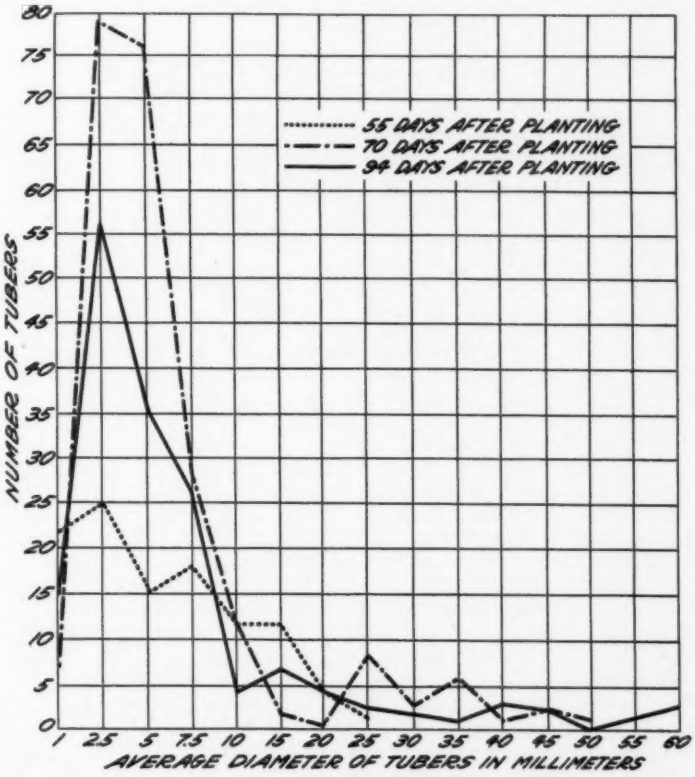


FIG. 4.—Graph showing the number of tubers at various diameters following three periodic removals of whole hills having three stems each. The majority of the tubers have remained 2.5 millimeters in diameter, indicating that the smaller tubers have not grown. The larger tubers have increased in size with each consecutive digging

as for instance, that the growing portion may remove nutritive materials that are necessary for the activity of the dormant parts. It seems plausible to assume that a rapidly growing tuber may inhibit the growth of other tubers in the vicinity. The stolons of these large tubers are scattered up and down the underground stem in such a manner that they are never far removed from the smaller nongrowing

ones. This point should be further investigated by removing some large tubers and determining whether the smaller ones would again resume growth.

RELATION OF GROWTH TO DISEASE

The preceding portion of this paper has discussed entirely matters concerning the development of the potato tuber. The remaining portion will deal principally with the relations existing between the activities previously described and infection of the potato tuber by *Actinomyces scabies*. Especial consideration will be given to the relation of the presence of stomata and the method of lenticel formation to scab susceptibility and infection.

The organism used for all inoculation work was isolated in 1918 by McKinney (*9*) from scabby tubers grown in Door County, Wis. The pathogenicity of the organism has been thoroughly established in a series of greenhouse and field experiments conducted at the University of Wisconsin during the last three years (*9*). Its morphology is the same as that described by Thaxter (*17*) and Drechsler (*5*), and it is therefore recognized as belonging to the species *Actinomyce scabies* (Thaxter) Güssow. The potatoes used were of the Irish Cobbler variety secured from the Spooner Branch Experiment Station, Spooner, Wis.

METHOD OF STUDY

During the course of the experiments the soil temperature was kept at 21° C., which temperature was maintained by the use of the Wisconsin soil temperature tanks (*8*), and the moisture held at 60 per cent of the moisture-holding capacity of the soil. The moisture content was regulated by means of biweekly weighings, and at each weighing water as needed was added through a glass tube inserted in the center of the soil at a depth of about 4 inches. In earlier tests (*9*), it was found that a soil held at 21° C. and having its moisture at 60 per cent of the moisture-holding capacity was favorable both for tuber and for scab development.

The most important part of this work was done by a periodic inoculation process, a method which enables one to inoculate growing tubers at any stage of their development without disturbing them. The seed piece was planted in galvanized iron pots 6 inches in diameter and 10 inches high. An inoculation apparatus was inserted in the can before the soil and seed piece were put in (fig. 5). This apparatus consisted of a hollow spiral lead pipe, the lower end of which was closed and the upper end open. The spirals of the pipe were of such a diameter that when the whole coil was placed in the can it lay snugly against the wall. The upper open end of the coil was allowed to protrude so that the inoculum might be introduced into it easily. The inoculum introduced into the coil was allowed to escape into the soil through small openings at intervals of 1½ inches apart made through the surface of the spiral which faced its axis. The coil was so placed that it surrounded that portion of the soil in which new tubers were formed. The soil used was a mixture of virgin soil and sand. The bottom 3 inches of the can were filled with virgin soil upon which the seed piece was placed,

and the next 5 inches with coarse and fine sand, or a mixture of sand and soil in various proportions. The purpose in using sand was to allow an even distribution of the spores through the soil, for soil of a fine texture seemingly retains the spores and prevents their distribution. All of the sand and soil, as well as the lead pipe and cans, were sterilized in an autoclave for four hours at a pressure of 15 pounds. The lead pipes were coated with paraffin after sterilization to guard against any toxic action which the lead might have on the fungus.

The thoroughness of the spore distribution through the soil was demonstrated by sterilizing a can of soil, in which the lead pipe had been placed, then forcing a spore suspension through the pipe into the soil according to the method described later. Nine portions of the soil, 0.5 cubic centimeter each, were removed under aseptic conditions from the center of the soil mass at depth intervals

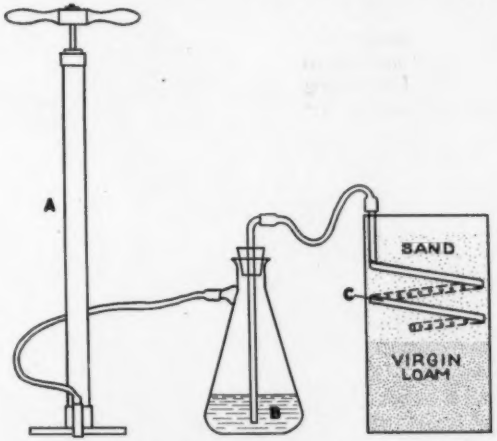
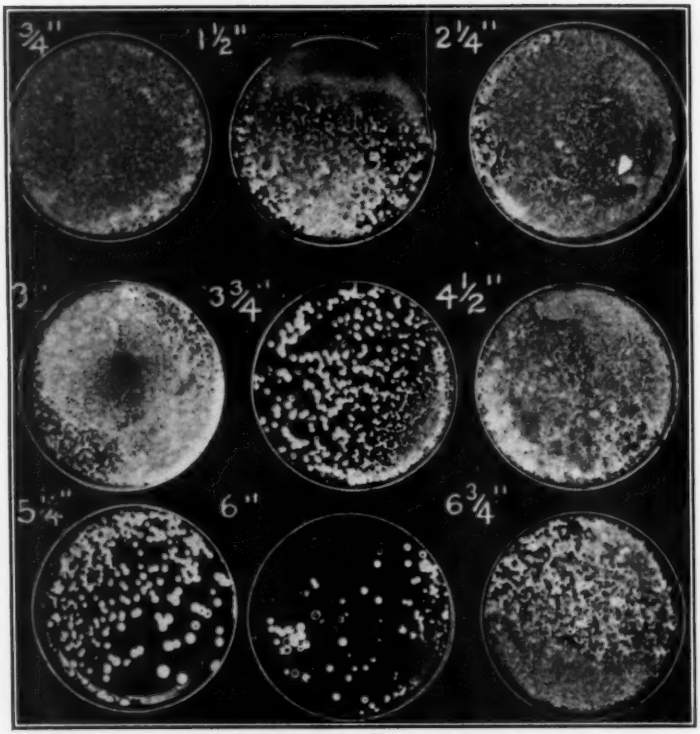


FIG. 5.—Diagram of the apparatus used for inoculating the sand which surrounded growing tubers. The inoculation was brought about without disturbing these tubers. A, large automobile tire pump; B, spores suspension contained in a side-neck filter flask; C, spiral lead pipe surrounding the growing tubers. The holes in this pipe allow a uniform distribution of the spores through the sand in which the tubers are growing.

of three-fourths of an inch. Each portion removed was placed in 5 cubic centimeters of sterile water and thoroughly mixed with it. One cubic centimeter of the mixture was poured on the surface of sterile agar in Petri dishes. Plate 3 shows the abundance of the organism in 0.1 cubic centimeter of soil at varying depths. The spore distribution certainly seemed thorough enough to reach all portions of the growing tubers.

In order to secure the organism in abundance for inoculation, it was grown on the surface of potato-dextrose agar in Petri dishes. When sporulation occurred copiously the surface layer was scraped off and made into a spore suspension. Each pot was inoculated with a suspension from the spores of three Petri dishes placed in 300 cubic centimeters of water. After the normal water content of the soil had become somewhat diminished, the 300 cubic centimeters of spore suspension was applied. A rubber tube was attached



The distribution of *Actinomyces scabies* spores through the soil in 10 by 6-inch cans, secured by the spiral pipe method. (See fig. 5.) Samples were taken at ¾-inch intervals from the center of the soil, beginning at ¾ inch depth and continuing to 6¾ inch depth. The colonies on each plate were secured from $\frac{1}{10}$ c.c. of soil. The numbers at the upper left-hand portion of the plates refer to the depth in inches at which the sample was taken

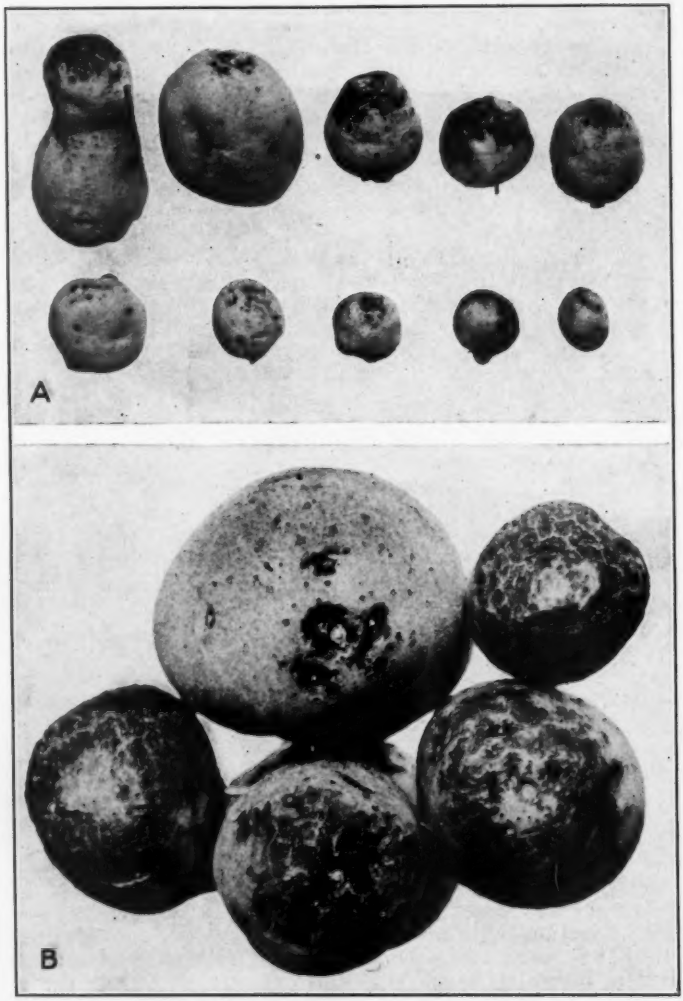
to the projecting end of the lead pipe and connected at the other end to a glass tube which extended through the cork to the bottom of a side-neck filter flask (fig. 5). The side neck of the flask was, in turn, connected by means of a rubber tube to a large automobile pump. The spore suspension was placed in the flask and when the pump was operated it was forced into the lead pipe out through the small holes into the soil. When the young tubers had been set for a short time, a certain number of the pots was inoculated, and at varying intervals of tuber growth, other pots were inoculated. Observations were made on the tubers to determine the length of time required for the disease to develop, the portion of the tuber infected, the place of infection, and the condition of the protective covering.

The experiment in which it was found that tubers set at about the same time, some growing and others remaining relatively stationary, suggested that there should be an inquiry into the relative amount of disease on tubers of different behavior. Seed pieces were therefore planted in 24 cans containing preinoculated soil. Previous to the inoculation, the soil was spread in a thin layer on a concrete floor and a spore suspension of *Actinomyces scabies* was sprinkled over it, the two being occasionally stirred thoroughly. Before this was done, however, the soil had been allowed to dry for some time, and the amount of water used in the suspension was of the right quantity to bring it to the required water content. The tubers were grown at a constant soil temperature of 21° C., and in soil kept at 60 per cent of its water-holding capacity. The relative amount of disease was determined by the number of tubers showing lesions.

In addition to the greenhouse experiments, others were performed in the field to discover whether or not the organism would infect tubers which were growing, but were forming no new epidermis, stomata, or lenticels. After the tubers had nearly reached maturity but were still growing, the soil was removed from around them, and they were marked with India ink and inoculated with a spore suspension. The soil which was replaced upon the tubers was also thoroughly saturated with the suspension. After the tubers had grown, as was determined by the India-ink test, they were dug and observations were made for the presence of the disease and the nature of the tuber growth, especial attention being paid to the condition of the protective covering in all parts of the tuber.

If the development of the disease depends on growing tissues, then the development of the lesion should give some indication of this fact. To determine if any such relation existed, the place of infection and the development of the lesion were carefully observed. Free-hand sections were made of lesions in several stages of development. These were made in an unstained and unkilled condition, so that the browning produced by the organism on the cells might be retained. This browning is lost entirely in the process of killing and embedding, which makes it difficult to trace the effects on the organism when it is located at some distance from the mycelium.

In order to determine if infection could occur on tubers of various sizes which were not growing, 10 tubers, averaging from 8 to 50 millimeters in diameter, were removed from plants and placed in a moist chamber, where they were immediately inoculated with a spore suspension of *Actinomyces scabies*. The surface protective covering varied among the several tubers. Some had an epidermis at the



Results of inoculating tubers with *Actinomyces scabies* after they have reached considerable size. The inoculation was accomplished by means of a special apparatus which prevented any disturbance of the tubers. (See fig. 5)

A.—Side view showing apical-end infection

B.—View of the apical end showing the scabbed portion surrounding a small spot where the disease has not yet become visible to the unaided eye. A microscopic examination showed that the lenticels and stomata of this region were infected

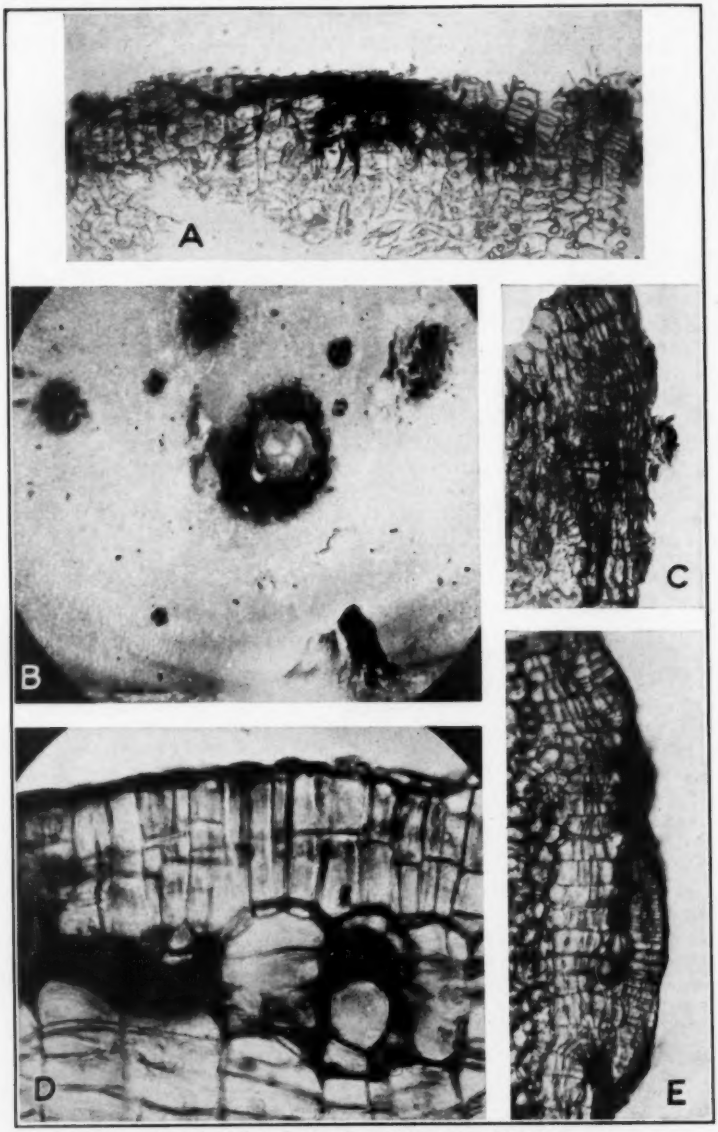
apical end with stomata and new lenticels present, whereas others were entirely covered with cork and had only mature lenticels. The spore suspension was sprayed on the surface of the tubers with an atomizer every other day for a period of two weeks. In no case did infection occur.

RESULTS OF PERIODIC INOCULATIONS

EXPERIMENT NO. 1.—The seed potatoes were planted on April 14, 1922, according to the manner described under "Methods." The first inoculation was made on June 3, 1922, in six cans. Three of these contained pure sand and three contained three-fourths sand and one-fourth soil. The tubers were removed from the six cans 10 days later and the following observations were made: Of a total of 104 tubers removed, the size of which ranged from 46 by 36 millimeters to less than 1 by 1 millimeter, 21 were infected. In no case was a tuber smaller than 10 by 10 millimeters infected, although there were 77 tubers, 80 per cent of the total, smaller than this. The infection was very abundant wherever it occurred, but in every case it was found on the apical end only (pl. 4, A). A noticeable infection of the lenticels occurred in the immediate vicinity of the eyes and the scale surrounding the bud of the eye (pl. 5, B). In a very small area in the region of the fastest growth, i. e., the vicinity of the terminal bud, there was a spot, variable in size, in which no infection was visible to the naked eye (pl. 4, B). A microscopic examination revealed, however, that the stomata there were infected, but the infection was so recent that it had not yet had time to become appreciable to the naked eye. The controls were free from disease.

EXPERIMENT NO. 2.—The potatoes used in experiment 2 were planted at the same time and in the same manner as those in experiment 1. On June 20, 1922, four pots containing one-half sand and one-half soil, and two pots containing three-fourths sand and one-fourth soil, a total of six pots, were inoculated. The removal was made nine days after the inoculation. The main results obtained were very similar to those in experiment 1, that is, all infections were at the apical end and the small tubers were not infected. It should be mentioned here that all of the large tubers in this case were also free from disease. The possible reason for this will be discussed later. The size of the tubers ranged from 50 by 44 millimeters to those smaller than 1 by 1 millimeter. The largest infected tuber was 33 by 23 millimeters. The total number of tubers was 96, the total number infected was 9, that is, 8 per cent of the total. The points to be considered here are that infections occurred only at the apical end and that neither the small nor the large tubers were infected.

The condition of the external protective tissue in various parts of the tubers as found in the two experiments previously described needs further consideration. As regards the protective tissue in the large, uninfected tubers, this was found to be cork, which covered the entire tuber. The stomata were all transformed into lenticels. With respect to the smaller uninfected tubers, various conditions were found. In most cases the tubers were completely corked, with lenticels present on the extreme apical end. In other small tubers,



Unstained photomicrographs of various phases of infection

A.—Cross section through a young lesion. The blackened parts show that the infection has occurred at the surface and has then extended to the interior among the subepidermal and parenchyma cells

B.—An enlarged photograph showing infection of the scale surrounding the compound bud of an eye and the lenticels in the vicinity. The crescent shaped darkening near the center shows the infection of the scale and the other rounded dark spots near by show lenticel infection

C.—Cross section of the margin of a lesion as seen beneath the surface, showing the spread of the disease along the phellogen region beneath the corky layer. The external portion of the lesion is to the right and out of the photograph

D.—Effects of the organism through the middle lamella of dividing cells in the subepidermal region. Enlargement taken from Plate 5, E. No mycelium is present in this region

E.—Young lesion demonstrating the spread of the disease from the surface portion of the lesion between the dividing subepidermal cells

the basal end was covered with cork, and the apical end with an epidermis. In these instances the outer portion of the epidermal cell walls were heavily cutinized, much more so than in the case of epidermal cells which cover rapidly growing tissues. This would indicate that growth had ceased in these small tubers. The apical

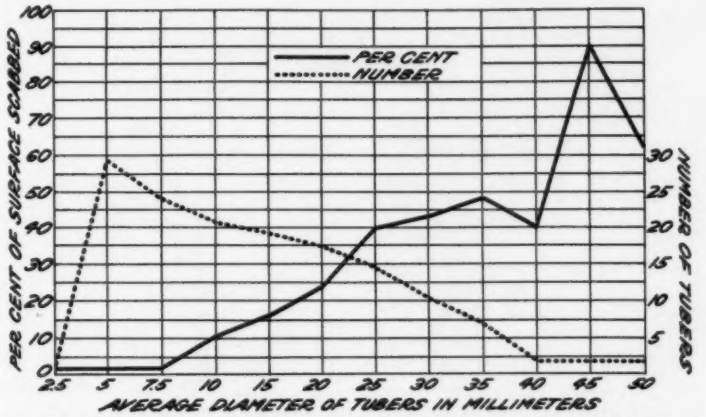


FIG. 6.—Graph showing the percentage of surface scabbed on tubers of various diameters and the number of tubers at each diameter. The percentage of surface scabbed as represented by the continuous line is greater on the tuber of the larger diameter. The tubers represented here were taken from 24 hills of potatoes the tubers of which had made their entire development in the presence of *Actinomyces scabies*

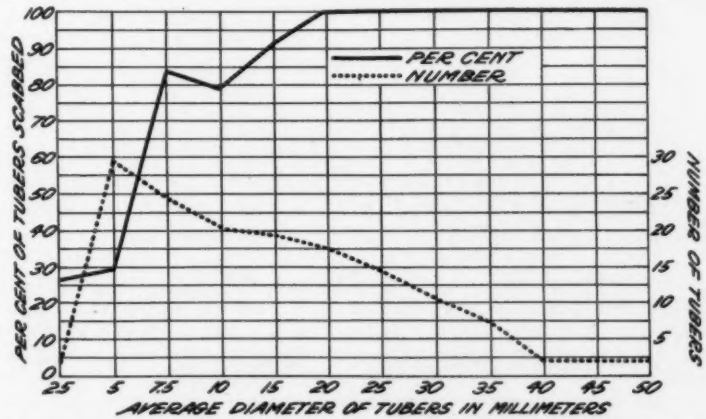


FIG. 7.—Graph showing the percentage of tubers of various diameters which have become diseased and the number of tubers at each diameter. The percentage of tubers scabbed as represented by the continuous line is greater among tubers of larger diameters. The tubers represented here were taken from 24 hills the tubers of which had made their entire development in the presence of *Actinomyces scabies*

end of infected tubers was covered with an epidermal layer in which were found stomata and newly formed lenticels.

In the case of tubers grown in preinoculated soil, there was a great difference in the percentage of the surface scabbed in the large and the small tubers. This was even more pronounced when one considers the percentage of tubers scabbed rather than the percentage

of surface scabbed. On the basis of the percentage of surface scabbed, if one compares tubers having an average diameter of 5 millimeters to those having an average diameter of 35 millimeters, it will be found that the percentage varies from 2 to 51 (fig. 6). On the basis of the percentage of tubers scabbed, considering the same sizes of tubers mentioned above, the percentage ranges from 25 to 100 (fig. 7). All of the tubers from 20 millimeters in diameter to the largest were infected. The reason why tubers smaller than 5 millimeters and larger than 35 millimeters were not considered is that the population was not thought large enough to be representative. The omitted ones, however, showed the same tendency as the others.

RESULTS OF FIELD INOCULATIONS

In the case where tubers were inoculated in the field no disease resulted either on the growing or on the nongrowing tubers. Field work presents the great difficulty that after the tubers are dug around, a large number of them fail to grow. This failure may be accounted for in two ways. At the point where the stolon of a tuber joins the underground stem there are a large number of roots united to the underground stem in such a way that their points of attachment partially surround the point where the stolon is attached at the node. This makes it difficult to remove the soil from around a tuber without destroying the roots, not only of those having their origin near the stolon, but also of some arising from other nodes. On the other hand, it might be that the particular tuber under observation had already reached its maximum size and would not have grown any more even if allowed to remain undisturbed. In all, 40 tubers were inoculated and marked with India ink to determine whether or not they grew. Of this number, 14 increased in size. On examination it was found that the method of growth was simply by a swelling of the tuber without the formation of any new epidermis or stomata at the apical end, a condition contrary to that found in younger growing tubers. It should be remembered, however, that the tubers used in this experiment were nearly mature, as was mentioned under "Methods."

POINT OF INFECTION AND FORMATION OF LESIONS

In order to determine with any degree of certainty the point of infection, it is necessary to detect the infection in its very early stages, for if it has started in the stoma or lenticel, and progressed for any apprecialbe time, all signs of these organs will be obliterated. Wherever it was possible to distinguish the point of infection, it was found in a lenticel or stoma, except where infection occurred near an eye, in which case it was always in a region where the protective layer was epidermis and stomata were present in the vicinity. Lutman (*13*) claims that the principal method of infection is through lenticels, but that it can also take place through the corky layer. As mentioned before, one must detect the infection very early or he will be deceived as to its exact location. A surface view of a newly infected lenticel usually shows a few darkened cells all around the periphery of the lenticel; in some cases the lesion may start at one edge and extend out from the side in a more

or less rounded form. This darkened area continues from the margin of the lenticel out under the external corky covering. One can see from the surface view the somewhat transparent corky layer over the darkened tissue beneath. A newly infected stoma appears normal as far as shape of the guard cells is concerned, but a light brownish color may be noted in the opening of it, and this extends along the margin of the guard cells and the cells adjacent.

A critical examination of fresh, unstained sections beneath the surface of the tissue immediately next to a young lesion reveals some interesting details of the manner in which the disease spreads from the original point of infection. The phellogen and subepidermal cells, which are regions of rapid cell division, especially during the formation of new cork, are blackened for some distance from the lesion as seen from the surface (pl. 5, A, C, E). A minute inspection of the details of the blackened cells shows that the extension takes place through the middle lamella. At the angles where four cells have come in contact they separate from one another and the space between soon becomes filled with a dark-brown liquid. A dark-brown color extends out along the middle lamella, which becomes abnormally thickened (pl. 5, B). Occasionally one finds a whole cell blackened, a condition which is caused perhaps by the darkening of the middle lamella of the wall which presents its face toward the observer.

A considerable amount of search has failed to reveal threads of the mycelium in the vicinity of the blackened tissue, a fact which might lead one to believe that the causal organism affects cells at a considerable distance. Bolley (*2*) claims that he found the mycelial threads of *Actinomyces scabies* in the diseased tissues. Others have stated that they have seen short, rodlike bodies present in the cells, and these they have believed to be broken fragments of the mycelium. In no case does the literature state whether or not the specimens were artificially inoculated in sterile soil. The writer has been able to find abundant filaments resembling those of *A. scabies* in the cells of field specimens which were not artificially inoculated. The filaments were not found in living cells but in dead corky ones near the surface of lesions. The lesions were about 5 millimeters or more in diameter. The invaded cells were not empty of food, for starch grains were present. Conclusions should not be drawn from field material, however, for there are many species of Actinomyces in the soil which may penetrate the dead or dying tissue and thus deceive the observer.

Freshly dug diseased tubers show the organism on the surfaces of lesions as a white hyphal covering. This may occur on field or on artificially inoculated specimens. Since the organism has not been found in the cells of diseased tissue, it must live at the surface and extend its influence beneath by means of an enzyme or toxin. The question as to the exact manner by which the organism secures nourishment has not been satisfactorily answered by these studies. It would be at variance with the known physiology of fungi to suggest that suberin might be utilized for nourishment. The walls of the corky cells in a scab lesion disintegrate and allow the cells to slough away, and by this process may permit the organism to gain access to starch grains and old dried cell contents which are found in corky cells.

DISCUSSION

The relation of disease and infection to the behavior of the tuber during the various phases of its life history needs further explanation. The most important points to be considered are: Infection of the tuber at the apical end; relation of tuber growth to disease; and period of susceptibility to infection.

Rapidly growing tubers which were inoculated became diseased only on the apical end, an indication that the conditions which exist in the external protective covering at the growing apical end are necessary for infection. Some of the conditions which have been noted at the time of infection are the presence of an epidermis, or an incompletely formed corky layer, and the formation of natural openings in these coverings. Since infection has always occurred either in stomata or in lenticels while they were in the process of formation, it is reasonable to suppose that these openings are necessary for infection. Stomata and lenticels are formed only in new and rapidly growing protective tissue which might lead to the conclusion that the presence of an epidermis with the formation of such organs in it is the chief factor necessary for infection.

The condition of the lenticels in the apical end of a tuber at the time of infection may predispose the tuber to infection in that region. When the lenticels first break open, the parenchyma cells below are freely exposed. This enables the organism or its products to come into direct contact with these cells. The parenchyma cells thus exposed have not been found to be suberized.

The results obtained in a portion of the experiment suggest that growth is essential for infection. Large tubers exposed to the scab organism during their entire life history showed much greater infection than smaller ones of the same age under the same conditions. Another experiment indicated that the large tubers grew and the smaller ones remained relatively stationary. It appears therefore that growth and disease are coincident.

The inoculation of entire hills of tubers at various periods in their life history resulted in a varying percentage of disease on tubers of different sizes, the larger ones showing some infection and the smaller ones none. There is evidence, as indicated above, that the large tubers were growing and the smaller ones were not. The idea that the occurrence of the disease depends on growth or the ability of a tissue to resume growth readily, is further strengthened by the fact that the tissue in the vicinity of the eyes is often infected. This tissue is able to resume growth very readily. The knobby outgrowths frequently observed on tubers are initiated at the eyes, a fact which shows that this region responds to the stimuli which cause cell division.

There are instances where growth occurs without liability to infection. None, for example, has been observed at the basal end of rapidly growing tubers when inoculated with *Actinomyces scabies*. It often happens that no infection occurs anywhere on the surface of growing tubers, and it never occurs on those not growing.

The condition existing in the external coverings of the tuber at the points where infection failed to occur must, therefore, prevent infection. The protective covering over the immune areas of growing tubers has always been found to consist of several layers of

corky cells similar to those on a mature tuber. Mature lenticels occur in the corky protective coat, but no stomata. The condition of the lenticels prevents infection occurring through them. This lack of infection in mature lenticels may be accounted for by the fact that the tissue covered by the lenticels is protected by a layer of suberize cells. The protective layer in the external portion of the lenticel is several cells in thickness. These facts show that increase in size alone will not predispose the tuber to disease, although it is coincident, in many cases, with the production of the disease. Nongrowing tubers have never become infected when inoculated, even though portions of their surface were covered with an epidermis containing lenticels and stomata. Thus it would seem that enlargement is essential to the production of the disease.

The development of a scab lesion indicates that the presence of dividing cells, or cells readily stimulated to division, aid in the production of the disease. The pathogen first affects the host by extending its influence through the middle lamella of the subepidermal cells. Later, when the host is more mature, it extends through the phellogen. The cells of the subepidermal layer are in a state of active division when the new corky layer is being formed, and at this time infection is very abundant. The cells of the phellogen divide to form new additions to the corky layer. Cell division in the phellogen is easily initiated at any time that stimulating agents are applied. *Actinomyces scabies* stimulates cell division, as is indicated by the type of disease which it produces, for this consists in an abnormally large production of corky cells.

It appears from the experimental evidence at hand that tubers of the size found on the market have at some time in their life history passed through a period of susceptibility, which was later followed by a period of immunity. The period of susceptibility may begin at any time in the growth of the tuber, provided it is forming a new epidermis with stomata and lenticels. The period of sus ceptibility ceases in growing tubers when the latter are no longer forming an epidermis with stomata or young lenticels, and also in any tuber when it has ceased to grow.

SUMMARY

Recent investigations of potato scab have given evidence that the development of the disease is intimately connected with the growth of the tuber.

Experiments conducted to determine the method of tuber enlargement showed that enlargement varied with the variety. Tubers of varieties that were normally elongated increased in length more rapidly than in width, whereas those that were normally round increased rather uniformly in all diameters.

The apical end of a rapidly growing tuber was found to be the region of greatest growth activity. This portion of the tuber was covered with an epidermis in which stomata were being formed. These stomata later were transformed into lenticels by a breaking open of the epidermis or of the new and incompletely formed corky layer in which the stomata were present. This rupturing of the protective covering freely exposed the parenchyma cells below the lenticels.

The epidermal and subepidermal cells covering the apical end of a growing tuber became transformed into several layers of corky cells by periclinal cell divisions, followed or accompanied by the suberization of the cell walls. The cells of the lenticels present in the corky layer also became suberized, thus protecting the parenchyma cells below.

Tubers produced by a single plant are initiated at nearly the same time. Some of these grow; others remain of approximately the same size. The tubers which remain small often disintegrate, so that a mature plant has ordinarily fewer tubers than a younger one.

The organism used for inoculation was a strain of *Acitinomyces scabies* (Thaxter) Güssow isolated from scabby potatoes grown in Door County, Wis. Its pathogenicity had been thoroughly established before these experiments were undertaken. Its morphology corresponded to that given by Thaxter and Drechsler.

Whole hills of tubers were inoculated periodically at varying stages of maturity by a special apparatus which permitted inoculation without disturbing the tubers or plants. These tubers were grown at a soil temperature of 21° C., and the soil was kept at 60 per cent of its moisture-holding capacity.

Tubers which were inoculated when the plants were only partially mature showed two types of behavior: (1) The large ones were infected at the apical end only and in the vicinity of buds situated near the apical end; (2) the small tubers were not infected.

Tubers which were inoculated when the plants were nearly mature showed three types of behavior: (1) The small tubers were not infected; (2) the medium-sized tubers were infected at the apical end only; (3) the largest tubers were not infected.

Individual tubers growing in the field, which were nearly mature when inoculated, did not become infected even though growth had not ceased.

In all cases where it was possible to determine the place of infection, it was found to be in a lenticel or in a stoma.

In every instance where tubers were infected as a result of artificial inoculation, the protective covering in the newly infected part was found to be epidermis or newly formed cork, in which stomata or young lenticels were being formed.

In every instance where large, nearly mature tubers failed to become infected when artificially inoculated, the entire, or nearly entire, external covering was found to be cork with no new natural openings being formed.

Small tubers which failed to become diseased when inoculated were either completely covered with cork or the basal end had a corky protective coat and the apical end a heavily cutinized epidermis with stomata and lenticels present. These tubers were not growing.

Histological studies of the lesion showed that the effect of the organism extends along the phellogen of the cells of the subepidermal layer by way of the middle lamella which was thickened and darkened. The phellogen and subepidermal layers are regions of rapid cell division in growing tubers.

The mycelium of the causal organism has not been found in lesions produced by artificial inoculation. The mycelium of a fungus resembling *Actinomyces scabies* has been found in lesions produced naturally in the field.

It seems that if the potato-scab disease is to occur, the tuber must be increasing in size, there must be stomata or young unsuberized lenticels present through which the infection can take place, and there must be dividing cells or cells which can easily be incited to division by the products of the organism, thus permitting the production of the typical corky scab lesions.

LITERATURE CITED

(1) ARTSCHWAGER, E. F.
1918. ANATOMY OF THE POTATO PLANT, WITH SPECIAL REFERENCE TO THE ONTOGENY OF THE VASCULAR SYSTEM. Jour. Agr. Research 14: 221–252, illus.

(2) BOLLEY, H. L.
1891. POTATO SCAB, AND POSSIBILITIES OF PREVENTION. N. Dak. Agr. Exp. Sta. Bul. 4: 1–14, illus.

(3) CHILD, C. M., and BELLAMY, A. W.
1919. PHYSIOLOGICAL ISOLATION BY LOW TEMPERATURE IN BRYOPHYLLUM AND OTHER PLANTS. Science (n. s.) 50: 362–365.

(4) CLARK, C. F.
1921. DEVELOPMENT OF TUBERS IN THE POTATO. U. S. Dept. Agr. Bul. 958, 27 p., illus.

(5) DRECHSLER, C.
1919. MORPHOLOGY OF THE GENUS ACTINOMYCES. I–II. Bot. Gaz. 67: 65–83, 147–168, illus.

(6) GÜSSOW, H. T.
1914. THE SYSTEMATIC POSITION OF THE ORGANISM OF THE COMMON POTATO SCAB. Science (n. s.) 39: 431–433.

(7) JONES, L. K.
1924. ANTHRACNOSE OF CANE FRUITS AND ITS CONTROL ON BLACK RASPBERRIES IN WISCONSIN. Wis. Agr. Exp. Sta. Research Bul. 59, 26 p., illus.

(8) JONES, L. R.
1921. EXPERIMENTAL WORK ON THE RELATION OF SOIL TEMPERATURE TO DISEASE IN PLANTS. Trans. Wis. Acad. Sci., Arts, and Letters 20: 433–459, illus.

(9) ——— McKINNEY, H. H., and FELLOWS, H.
1922. THE INFLUENCE OF SOIL TEMPERATURE ON POTATO SCAB. Wis. Agr. Exp. Sta. Research Bul. 53, 35 p., illus.

(10) JÜRGENS, R.
[1872]. ANATOMIE UND PHYSIOLOGIE DER KNOLLE VON SOLANUM TUBEROSUM IM ZUSTANDE DER WINTERRUHE UND DER KEIMUNG. 47 p. Berlin. (Inaug. Diss.)

(11) KEITT, G. W.
1922. THIRD PROGRESS REPORT ON APPLE SCAB AND ITS CONTROL IN WISCONSIN. (Abstract) Phytopathology 12: 54.

(12) LOEB, J.
1917. A QUANTITATIVE METHOD OF ASCERTAINING THE MECHANISM OF GROWTH AND OF INHIBITION OF GROWTH OF DORMANT BUDS. Science (n. s.) 45: 436–439.

(13) LUTMAN, B. F.
1913. THE PATHOLOGICAL ANATOMY OF POTATO SCAB. Phytopathology 3: 255–264, illus.

(14) ———
1919. RESISTANCE OF POTATO TUBERS TO SCAB. Vt. Agr. Exp. Sta. Bul. 215, 30 p., illus.

(15) MCCALLUM, W. B.
1905. REGENERATION IN PLANTS. I–II. Bot. Gaz. 40: 97–120, 241–263, illus.

(16) REED, T.
1910. ON THE ANATOMY OF SOME TUBERS. Ann. Bot. 24: 537–548.

(17) THAXTER, R.
1891. THE POTATO "SCAB." Conn. Agr. Exp. Sta. Ann. Rpt. 1890: 81–95, illus.

87856—26†——6

AN ANATOMIC STUDY OF ZONITOIDES ARBOREUS SAY[1]

By PAUL BARTSCH, *Curator, Division of Mollusks, U. S. National Museum*, and MARY ELAINE QUICK, *Instructor in Zoology, George Washington University*

INTRODUCTION

The recent findings by R. D. Rands, of the Office of Sugar-Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, that a small mollusk plays an important rôle in the sugar-cane-root disease in Louisiana, has made it desirable to ascertain beyond question of doubt the systematic position and identity of this organism.

In order to determine whether the mollusk found in the earthworm burrows about the roots of sugar cane (on which it feeds and thus admits infection with resulting decay of the root) belongs to the same species as that which frequents the more normal habitat in the same region, it was deemed desirable to make not only a close examination of the shell characters of both, but a minute anatomic analysis and a comparison with typical material from farther north. To settle this beyond doubt, three sets of specimens were subjected to minute examination of the shell and anatomic characters. These specimens were:

1.—Taken from under decaying bark of deadwood, at Washington, D. C.

2.—Taken from under decaying bark of deadwood, at Houma, La.

3.—Taken from earthworm burrows about the roots of sugar cane, at Houma, La.

The first are held to be typical *Zonitoides arboreus* Say, a species which was described by Thomas Say in 1817 in an article on Conchology in Nicholson's Encylopedia, First American edition, and figured therein on Plate 4, Figure 4, as *Helix arboreus*. No type locality is mentioned by Say, but it is more than likely that his specimens were obtained at Philadelphia, Pa., from which place many of the things described by him originated. The Washington specimens are absolutely identical in shell characters with the material from the Philadelphia region.

DISTRIBUTION, FOOD, AND REPRODUCTION

Zonitoides arboreus is a widely distributed species, extending practically over all of North America. The habitat in which it is most frequently found is under loose, moist bark of decaying trees, although any decaying wood or vegetable detritus seems to serve as a suitable place of abode. These animals are probably largely nocturnal in their habits. They may be found moving about in the open in wet weather, or on moist cloudy days during the warm part of the year. Sometimes they climb to considerable heights on the trunks of trees or ledges of rock. It is at such times

[1] Received for publication Aug. 7, 1925; issued March, 1926.

that they do their foraging. The food in such habitats consists largely of mycelial threads of fungi, as can easily be demonstrated by an analysis of the intestinal contents.

Mating is probably largely nocturnal. Most of the specimens found conjugating were discovered in the early morning on moist days. When conjugation takes place, one animal acts as male only and the other as female only. There is, therefore, not a reciprocal fertilization, although the animals are hermaphroditic. The eggs are deposited under dead, more or less moist bark, or similar situations, as small spherules, sometimes singly and at other times as small clumps; when in groups they are more or less agglutinated.

EXTERIOR OF ANIMAL

(Plate 1)

The entire surface of the animal appears to be invested in a transparent cuticle through which the subjacent tissues shine as an ashy gray layer on the upper surface. The tentacles are of the same color as the back, while the sides of the foot are of a much paler gray. The underside of the foot is flesh colored, with an ashy tinge. The eyes are black.

Plate 1 shows four poses, showing the animal from various angles, all much enlarged.

THE SHELL

(Plate 2)

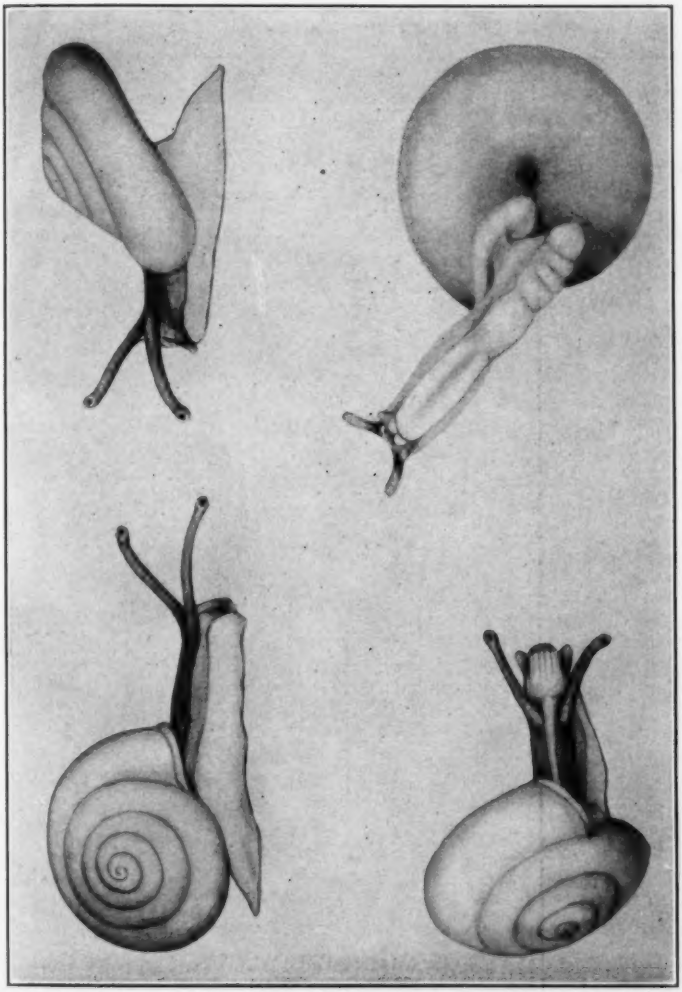
The shell is depressed helicoid, of 4½ to 5 whorls, thin, and horn-colored. The first turn or turn and one-third (nuclear whorls) are smooth and well rounded. The remaining turns are well rounded, appressed at the summit, the appressed portion appearing as a smooth spiral line at the summit. This part of the surface of the shell is marked by rather rough wrinkle-like lines of growth, and numerous, microscopic, closely spaced, spiral striations. Periphery of the last whorl well rounded. Base openly umbilicated, marked like the upper surface by rough, wrinklelike lines of growth and numerous spiral striations which are much coarser than those on the upper surface. Aperture oval, outer lip thin. The specimens shown on Plate 2 are:

A.—Cat. No. 335915, U. S. N. M., taken from under decaying bark of deadwood, at Washington, D. C., which measures: Greater diameter 4.7 mm.; lesser diameter 3.8 mm.; height 2.5 mm.

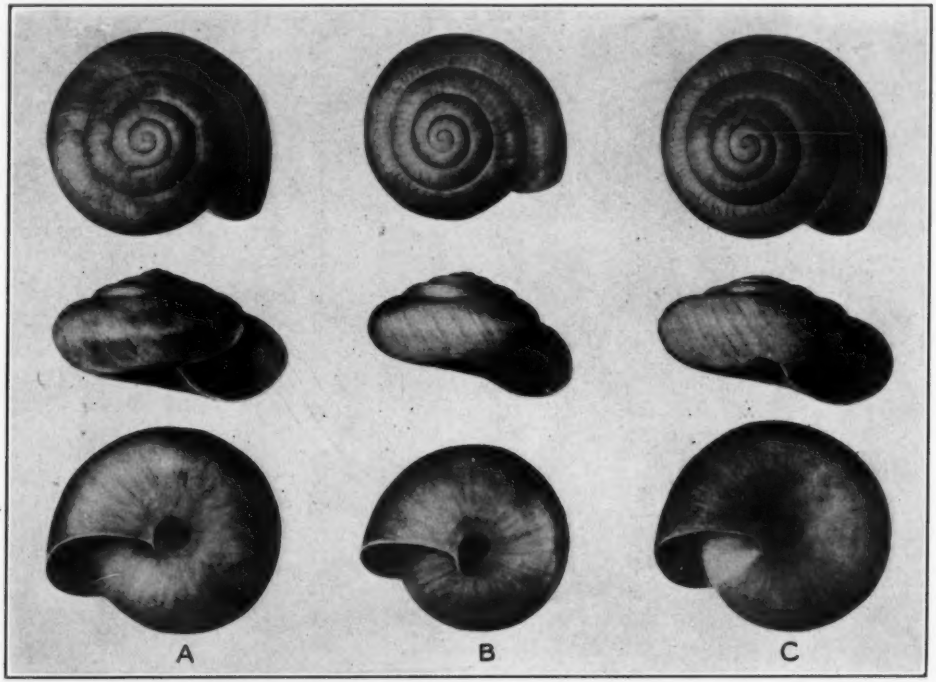
B.—Cat. No. 362712, U. S. N. M., taken from under decaying bark of deadwood, at Houma, La., which measures: Greater diameter 4.3 mm.; lesser diameter 3.4 mm.; height 2.4 mm.

C.—Cat. No. 362713, U. S. N. M., taken from earthworm burrows about the roots of sugar cane, at Houma, La., which measures: Greater diameter 4.5 mm.; lesser diameter 3.8 mm.; height 2.4 mm.

The variations in shell characters are not beyond the range of variation found in the shells in any one locality throughout the range of the species. No characters to which even a subspecific designation might be given were observed when a lot of southern specimens were compared with those of extreme northern and intermediate ranges.



Four drawings to show all sides of the expanded animal, made from a District of Columbia specimen. Much enlarged



A.—Three views of the shell of a specimen taken from a rotten log, in the District of Columbia
B.—Three views of the shell of a specimen taken from a rotten log, at Houma, La.
C.—Three views of the shell of a specimen taken from earthworm burrows in cane field, at Houma, La.

ANATOMY

(Plate 3, the three bottom drawings)

DIGESTIVE SYSTEM

The mouth (1) connects with the buccal cavity (2) by a moderately large, rather thick-walled tube. The buccal mass inclosing the buccal cavity is of triangular shape. From the upper angle of this mass the oesophagus (3) arises. The oesophagus, after passing forward, makes a sudden turn backward, this deflection being about twice the length of the first part. The oesophagus connects with the stomach (4), which has about three times the diameter of the oesophagus in its median portion and describes a rather open arc in continuation of the backward deflection of the oesophagus; at the pyloric end it makes a sudden turn as it passes into the intestine (5). The intestine receives the two hepatic ducts (6) at its anterior end. The intestine passes forward to the anterior angle of the oesophagus, and is then reflected backward for about one-third of its length, when it is again flexed forward in a curve that embraces two-fifths of its entire length. The salivary glands (7) occupy the region of the anterior flexure of the oesophagus and are rather inconspicuous. The hepato-pancreas is conspicuously packed about the whole of the digestive system. The buccal retractor (8) is attached to the basal portion of the buccal mass.

A series of specimens was dissected from each of the three localities mentioned in order to determine what range of variation they might present. The variations in each locality were trifling, as were the variations in the three localities when compared with each other. No distinctive features of any kind were noted in the digestive system of the material dissected.

The bottom row of drawings in Plate 3 shows the digestive systems of the three specimens, identified as follows:

A.—Taken from under decaying bark of deadwood, at Washington, D. C.

B.—Taken from under decaying bark of deadwood, at Houma, La.

C.—Taken from earthworm burrows about the roots of sugar cane, at Houma, La.

THE RADULA

(Plate 4)

The radula shows a central rachidian tooth, and five teeth that may be termed laterals, and 18 marginal teeth; in other words, 24 teeth from the center out to the lateral margins.

Plate 4 shows radulae from the three localities:

A.—Taken from under decaying bark of deadwood at Washington, D. C.

B.—Taken from under decaying bark of deadwood at Houma, La.

C.—Taken from earthworm burrows about the roots of sugar cane at Houma, La.

When the radulae from the three localities are carefully compared, no characters are found that may not be observed as variations in the rows of teeth belonging to any one of the single individuals.

REPRODUCTIVE SYSTEM

(Plate 3, the top row of figures)

The ovotestis (1) is an inconspicuous leaf-shaped organ appressed to the liver. The hermaphroditic duct (2) is slender and long and connects with the accessory gland (3), which is closely attached to the albumen gland (4), which heads the oviduct. The oviduct (5) is a rather large and conspicuous organ terminating in the vagina (6) posteriorly. The vas deferens (7) is a slender cord which is attached to the oviduct for a great part of its length, only the posterior fourth being free and this terminates in the penis sac (8). The dart sac (9) is about one and one-half times as long as the penis sac and bears the two inconspicuous coronal glands (10) at its summit. The vesicula seminalis (11) is a small organ joined to the vagina at about the anterior third by a very long duct. The cloaca (12) is a short thick-walled tube. The dart sac retractor muscle (13) connects the dart sac with the duct of the seminal vesicle at the base of the penis.

In the comparative study of the reproductive system of specimens from the three localities, no variations were observed that were not also noted in the examination of a series of specimens from each of the three localities.

The three top drawings of Plate 3 show the reproductive system of the three specimens, identified as follows:

A.—A specimen taken from under decaying bark of deadwood at Washington, D. C.

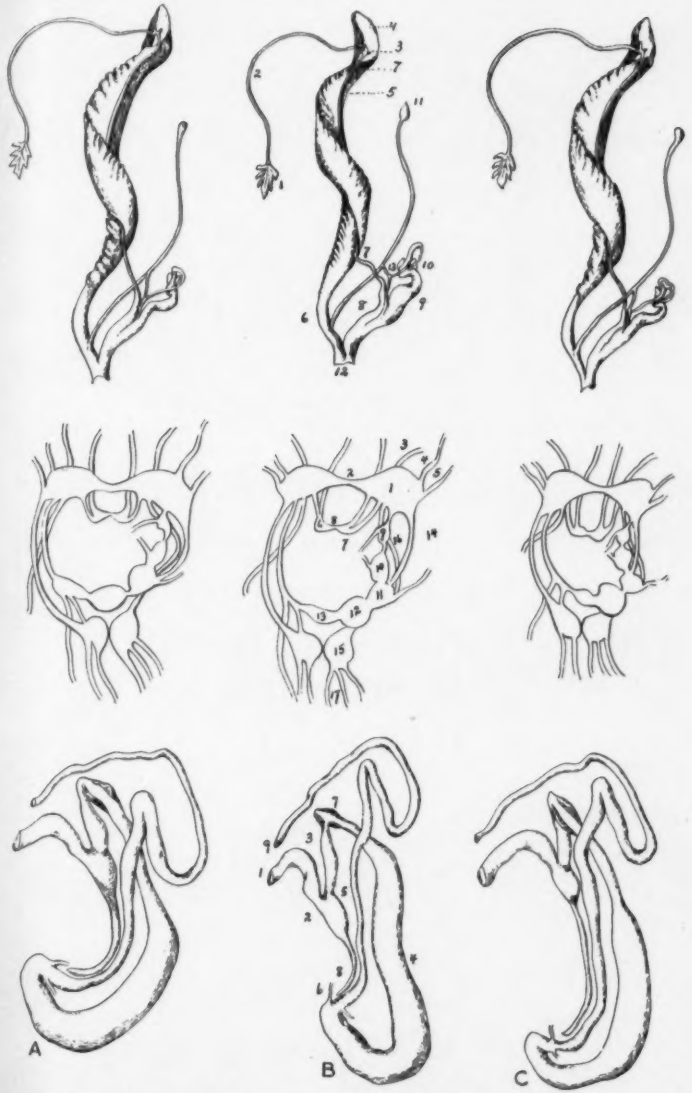
B.—A specimen taken from under decaying bark of deadwood at Houma, La.

C.—A specimen taken from earthworm burrows about the roots of sugar cane at Houma, La.

THE CENTRAL NERVOUS SYSTEM

(Plate 3 middle row of drawings)

The cerebral ganglia (1) are large, and are connected by the cerebral commissure (2). The cerebral ganglia give rise to the optic (3), tentacular (4), and sensory (5) nerves. The two buccal ganglia (6) are small, and connect with the cerebral ganglia by the cerebral buccal commissure (7) and with each other by the buccal commissure (8). Adjoining the cerebral ganglia is the parietal ganglion (9), which is about half the size of the right upper pleural ganglion (10), which lies immediately below it and connects with it. Adjoining the latter is the pleural ganglion (11), which is of about the same size as the upper right pleural ganglion. The left pleural ganglion (12) is about the same size as the right pleural ganglion, and connects with it. Completing the chain of five ganglia there is the abdominal ganglion (13), which is only about one-third the size of the pleural ganglion. The cerebral pleural commissure (14) connects the cerebral ganglion with the pleural ganglion. Below the chain of five ganglia are the two rather large pedal ganglia (15), which are connected with the cerebral ganglia by the cerebro-pedal commissure (16). The pedal ganglia give off a series of mantle nerves (17) basally.

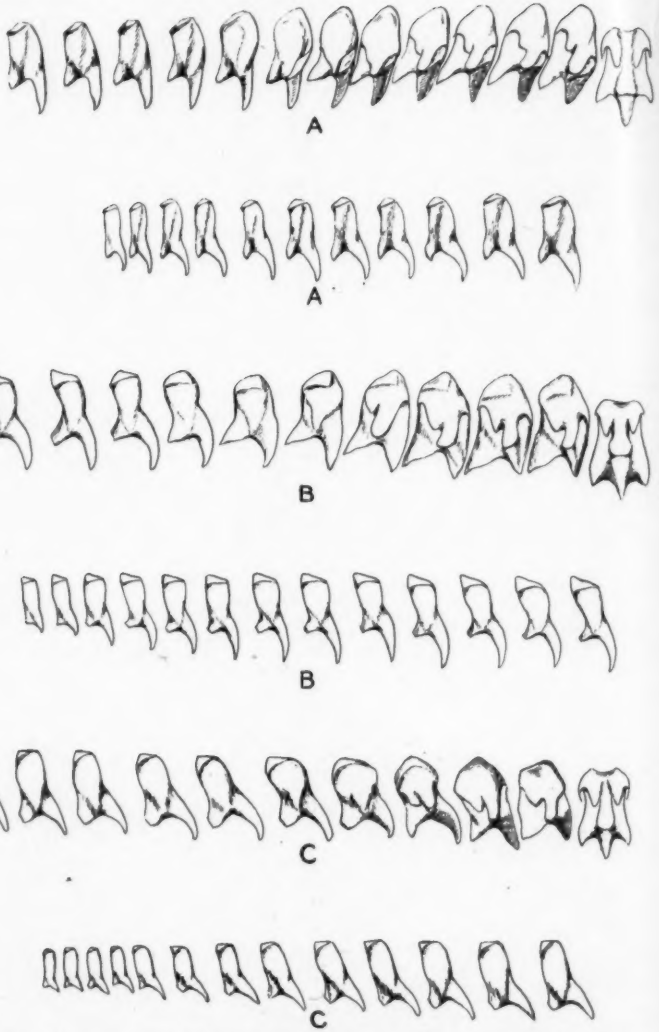


A.—Dissections of a specimen taken from a rotten log, in the District of Columbia
B.—Dissections of a specimen taken from a rotten log, at Houma, La.
C.—Dissections of a specimen taken from earthworm burrows in cane field, at Houma, La.

Reproductive system (top figures): 1, ovotestis; 2, hermaphroditic duct; 3, accessory gland; 4, albumen gland; 5, oviduct; 6, vagina; 7, vas deferens; 8, penis; 9, dart sac; 10, coronal gland; 11, seminal vesicle; 12, cloaca, 13, retractor dart sac muscle

Nervous system (middle figures): 1, cerebral ganglion; 2, cerebral commissure; 3, optic nerve; 4, tentacular nerve; 5, sensory nerve; 6, buccal ganglion; 7, cerebral buccal commissure; 8, buccal commissure; 9, parietal ganglion; 10, right upper pleural ganglion; 11, left upper pleural ganglion; 12, left pleural ganglion; 13, abdominal ganglion; 14, cerebro-pleural commissure; 15, pedal ganglion; 16, cerebro-pedal commissure; 17, mantle nerve

Digestive system (bottom figures): 1, mouth; 2, buccal body; 3, œsophagus; 4, stomach; 5, intestine; 6, hepatic duct; 7, salivary gland; 8, retractor buccal body; 9, anus



Radula studies showing the left half of a row of teeth taken from the median portion of the organ, the rachidian tooth being the right one of each series

A.—Radula from a specimen taken from a rotten log in the District of Columbia
B.—Radula from a specimen taken from a rotten log at Houma, La.
C.—Radula from a specimen taken from earthworm burrows in cane field at Houma, La.

As in the other systems and characters just reviewed, the central nervous system shows no distinguishing characters in the specimens dissected from the three localities. The middle row of drawings in Plate 3 show the central nervous systems, from specimens identified as follows:

A.—From a specimen taken from under decaying bark of deadwood at Washington, D. C.

B.—From a specimen taken from under decaying bark of deadwood at Houma, La.

C.—From a specimen taken from earthworm burrows about the roots of sugar cane at Houma, La.

SUMMARY

A comparison of specimens taken from—

Under decaying bark of deadwood at Washington, D. C.,

Under decaying bark of deadwood at Houma, La.,

Earthworm burrows about the roots of sugar cane at Houma, La., shows—

No observed differences in shell characters.

No observed differences in the structure of the digestive system.

No observed differences in the structure of the radula.

No observed differences in the structure of the reproductive system.

No observed differences in the structure of the central nervous system.

The writers therefore feel justified in considering as typical *Zonitoides arboreus* Say the organism now held responsible for much of the pitting and mutilation of sugar-cane roots in Louisiana, in spite of the unusual mode of feeding and assumed habitat.

STUDIES ON GOSSYPOL: THE GOSSYPOL AND D-GOSSYPOL CONTENT OF SOME NORTH CAROLINA COTTONSEED MEALS [1]

By F. W. SHERWOOD

Assistant Chemist in Nutrition Investigations, North Carolina Agricultural Experiment Station [2]

INTRODUCTION

In the manufacture of cottonseed meal, which is a by-product, the decorticated kernels are usually cooked before the oil is expressed, in order to increase the yield of oil. The details of the cooking vary to a considerable extent in different mills.

In North Carolina a few mills use the expeller or cold-press method, in which the decorticated raw kernels are usually dried in a preheater and are then passed into the expeller proper. In the expeller they are forced by pressure through small orifices, this operation subjecting them to a grinding action which squeezes out the oil. Both the dry heating and the grinding action are important and will be referred to later in connection with the *d*-gossypol content.

The majority of the mills use either the open-kettle or the continuous-cooker process. In the former method the cottonseed kernels are agitated in large steam-jacketed kettles until they are more or less thoroughly comminuted. The time of cooking and the steam pressure (temperature) in the jacket vary greatly. After cooking, the kernels go to the cake former and then to the hydraulic press, where the oil is expressed. The press cake is then ground to a fine meal.

The continuous cooker, which is often used in place of the open kettle, consists of three to five steam-jacketed drums or stacks placed one above the other. The raw kernels enter the upper stack and proceed downward as those below are emptied. There is considerable variation in the time required for the kernels to pass through the stacks and in the steam pressure used in the jackets at the different mills.

In either of these methods water or steam may be added or a current of air passed through the cooker, in order to regulate the moisture content of the kernels.

Withers and Carruth (*1*, *2*, *8*, *9*) [3] have shown that the cooking process exerts a profound reducing effect on the toxicity of the cottonseed, and that little if any gossypol can be extracted from the well-cooked cottonseed meal.

These investigators suggested that this lower toxicity is due largely to the oxidation of the gossypol to a less toxic substance which they called *d*-gossypol, and they found that the presence of water aids in this conversion.

---

[1] Received for publication July 14, 1925; issued March, 1926.

[2] This work was done in 1921, under the supervision of W. A. Withers, chemist, North Carolina Agricultural Experiment Station, now deceased. The writer wishes to acknowledge his indebtedness to Doctor Withers for his advice and interest; to J. O. Halverson for criticisms and aid in preparing the manuscript; and to the cottonseed-oil mills for information and samples which they supplied.

[3] Reference is made by number (italic) to "Literature cited," p. 800.

That the gossypol content of cottonseed tends to vary with the locality of growth was demonstrated by Schwartze and Alsberg (*5*, *6*). They found that this variation ranges from 0.4 to 1.2 per cent of the kernels, and that the seed from the Atlantic Coast States contain an average of a little less than 1.0 per cent of gossypol.

The work reported in this paper was undertaken in order to study the variations in the amount of gossypol and *d*-gossypol in North Carolina cottonseed meals and to determine whether it was possible to correlate these variations with the mill treatment to which the meal was subjected.

METHODS

Samples of cottonseed meal, together with certain information about the mill practice, were requested from each of the cottonseed-oil mills in North Carolina.

GOSSYPOL DETERMINATION

Fifty grams of the meal were extracted in a Soxhlet until the ether which siphoned over was colorless. One cubic centimeter of aniline was added to this extract, and the ether was evaporated. The residue was allowed to stand for one week, when the precipitated aniline gossypol was filtered on a tared Gooch crucible and washed with a small amount of ether and then thoroughly with petroleum ether. The crucible with contents was dried at 100° F. and weighed.

D-GOSSYPOL DETERMINATION

The ether-extracted residue from the gossypol determination was saturated with 100 cubic centimeters of aniline, and the mixture was heated for 5 minutes at 110° C., with constant stirring. It was immediately thrown on a Buchner funnel, and as much of the hot aniline as possible was removed by suction. When sufficiently cool the remaining aniline was washed out with ether. After the removal of the ether from the combined filtrate and washings the aniline was distilled until the residue had a volume of 5 to 10 cubic centimeters. This residue was washed into a small beaker with a few cubic centimeters of ether and allowed to stand for one week. The precipitated aniline *d*-gossypol was then filtered through a tared Gooch crucible and washed first with a small quantity of ether and then thoroughly with petroleum ether. After drying at 100° C. the crucible and its contents was weighed.

CONVERSION FACTOR

Carruth, from his analyses of aniline gossypol (*1*), obtained the factor 0.74 for the conversion of aniline gossypol into its gossypol equivalent. Since aniline *d*-gossypol contains approximately the same amount of nitrogen as aniline gossypol, as has been shown by Carruth (*2*) and by unpublished analyses in this laboratory, this factor (0.74) was used for obtaining both the gossypol and the *d*-gossypol equivalents of the respective aniline compounds.

SUMMARY OF INFORMATION SUPPLIED BY THE MILLS

Nineteen mills sent samples of their meal and gave more or less complete answers to a questionnaire. In some cases the replies seem to be incorrect, but they are presented in Table I as they were submitted. The color of the meal, shown in the last column of Table I, is the writer's observation.

TABLE I.—*Data concerning the cottonseed and manufacturing conditions*

OPEN KETTLE

Sample No.	Soil on which grown	Variety of cotton	Water added during cooking	Time of cooking (minutes)	Temperature of seed in cooker (° F.)	Steam pressure in jacket (pounds)	Color [a]
1	Sandy loam	Mixed	No	24	210		B
2	do	Cleveland Big Boll	Yes	48		60	YB
3		Mixed	Steam	12	300	90	Y
4	Black	do	No	20		50	YB
5	Red	do	No	25	210		Y
6	do	do	No	22	70		YB

CONTINUOUS COOKER

7	Loam	Mixed	No	[b] 18 (1)		40	YB
				18 (2)		60	
8	Sandy	do	No	20 (1)		80	Y
				13 (2)			
9	Coastal Plain	Short Staple	8 per cent	38	218	80 to 90	Y
10	Sandy loam	Cook's Improved	No	20 (1)	175	40	Y
				20 (2)	175	75	
				20 (3)	175	90	
11			2 per cent	20 (1)	170	90	YB
				20 (2)	200	60	
				20 (3)	215	40	
				20 (4)	220	20	
12	Norfolk loam	Mixed	5 per cent	20 (1)	180	75	DB
				20 (2)	200	60	
				20 (3)	220	50	
				20 (4)	220	30	
13			Steam	20 (1)		60	YB
				20 (2)		60	
				20 (3)		40	
				20 (4)		10	
14	Loam	Mixed	Small spray.	20 (1)	218 to 220	5	YB
				20 (2)		10	
				20 (3)		70	
				20 (4)		65	
15	Sandy	Improved Small Seed	Yes	23 (1)	220	60	YB
				23 (2)	220	60	
				23 (3)	220	60	
				23 (4)	220	25	
16	do	Mixed	No	7 (1)	64	100	YB
				7 (2)	79	100	
				7 (3)	100	100	
				7 (4)	107	100	
17	Clay	Mixed	No	20 (1)	210		YB
				20 (2)			
				20 (3)			
				20 (4)			
[c] 18			2 per cent	20 (1)	220		YB
				20 (2)			
				20 (3)			
				20 (4)			
				20 (5)			
[c] 19			do	20 (1)	220		YB
				20 (2)			
				20 (3)			
				20 (4)			
				20 (5)			

EXPELLER

20	Red and gray	Cleveland Big Boll					GY

All mills reported that the meals were made from good sound seed.

[a] The abbreviations used to denote color of the meals are: DB, very dark brown; B, brown, lighter than DB, but without a yellow tinge; YB, brownish yellow or yellowish brown; Y, bright yellow without a brown tinge; and GY, a very bright greenish yellow.

[b] The numbers in parentheses refer to the respective stack of the continuous cooker.

[c] Meals 18 and 19 were taken from the same mill. They contained 7 and 8 per cent NH_3, respectively.

RESULTS OF THE ANALYSES

The gossypol and *d*-gossypol contents of the meals which are described in Table I are presented in Table II, the figures being in most cases the average of two determinations.

TABLE II.—*Gossypol and d-gossypol content of cottonseed meals (1921)*

OPEN KETTLE

Sample No.	Per cent moisture	Per cent on dry basis		Sample No.	Per cent moisture	Per cent on dry basis	
		Gossypol	D-Gossypol			Gossypol	D-Gossypol
1	4.92	[a]0.045	0.874	4	7.52	0.019	0.919
2	6.66	.154	.755	5	6.36	.021	.881
3	6.32	.160	.669	6	6.66	.143	.963

CONTINUOUS COOKER

7	7.58	0.034	0.902	14	5.83	0.025	0.820
8	8.08	.228	.789	15	5.61	[a].329	1.014
9	6.43	.048	.843	16	5.21	.020	.754
10	5.74	[a].022	.848	17	6.04	.026	1.019
11	6.20	[a].023	.811	18	7.68	.010	.860
12	5.72	.016	.808	19	7.75	.009	.878
13	7.17	.040	.773				

EXPELLER				RAW KERNELS			
20	5.06	0.102	0.505			0.58	0.14

[a] The precipitate consisted of a dark-gray amorphous material instead of the usual orange-colored crystals of aniline gossypol.

ADDITIONAL GOSSYPOL AND D-GOSSYPOL DETERMINATIONS IN COTTONSEED MEAL

In 1918 L. F. Williams and S. J. Marion, while working in this laboratory, determined the gossypol and *d*-gossypol content of a number of cottonseed meals which they had obtained from various oil mills in North Carolina. Their results, together with the available information concerning the manufacturing processes, are given in Table III.

TABLE III.—*Gossypol and d-gossypol in cottonseed meal (1918)*

Sample No.	Cooking time (minutes)	Temperature in cooker (°F.)	Steam pressure in jackets (pounds)	Per cent		
				Moisture	Gossypol	D-Gossypol
OPEN KETTLE						
21	40	135–250		7.75	0.033	0.910
22	33		60	7.38	.051	.758
23	20	202	60	8.29	.111	.858
24	22½		40	10.35	.078	.544
25	10–12		80	9.73	.152	.584
26	20		60–80	6.97	.089	.575
27	20			8.01	.078	.932
28	20		40	7.74	.089	.754
CONTINUOUS COOKER						
29	22	240	25	8.46	0.037	1.076
30	60	210	5–45	8.30	.058	.832
31	24		15–35	10.12	.044	.696
32	120	226	35	12.05	.062	.984
33	72	212–215	15–20	8.10	.010	.751
34	27		50	9.05	.018	.843
35	96		25–100	10.60	.058	.633
36	27		50	8.09	.007	.999
37	75	200–230	30–60	7.21	.007	.888
EXPELLER						
38		162		7.18	0.026	0.333
39		166–170		7.54	.020	.425
40		160		6.39	.023	.348
MISCELLANEOUS						
41	Cooked meals from mill which made meal, 32.			6.35	0.081	0.566
42	Cooked meals from mill which made meal, 22.			6.31	.033	.470
43	Raw meals			8.38	.559	.000

DISCUSSION

The analytical methods employed for the determination of gossypol and *d*-gossypol have not been developed to give the degree of accuracy that may be desired. However, the results presented in the above tables are comparative, and important assumptions can be drawn from them in regard to the probable toxicity of the meals for feeding purposes as well as in regard to the amounts of gossypol and *d*-gossypol present.

The results clearly show that the greater part of the gossypol originally present in the raw cottonseed is converted into *d*-gossypol by the manufacturing processes used in expressing the oil. This confirms the observations of Withers and Carruth (*1*, *2*, *8*, *9*).

No direct studies have been made on the pharmacology or toxicity of *d*-gossypol, so far as the writer knows. Withers and Carruth (*9*, *p. 449*) summarize their indirect evidence on the toxicity in the following words:

> Cooking the [cottonseed] kernels under oil-mill conditions causes a profound reduction in toxicity. This change is so great that the thoroughly cooked products show no pronounced toxic effect on rats and poultry in suitable diets. Thoroughly cooked meals, however, appear to be definitely injurious to rabbits and swine, * * *.

If *d*-gossypol is only slightly or not at all toxic (*7*), as seems probable, the discrepant results of various investigators on the toxicity of

cottonseed meal may be explained by the variable though small gossypol content of the different lots of cottonseed meal with which they worked.

In this connection it is interesting to note that under the experimental conditions used by Schwartze and Alsberg (*6*) the threshhold value of toxicity of gossypol was close to 0.0675 per cent of the total diet. The analyses reported in Tables II and III show that only 11 of the 40 meals examined contained this amount of gossypol. If any of these meals constituted as much as 50 per cent of a diet similar to that used by Schwartze and Alsberg in their feeding experiments, only 5 of them (12.5 per cent) would contain sufficient gossypol to cause injury to the white rat.

Another possible explanation of the varying results of different investigations is that in some cases the resultant symptoms obtained in feeding experiments with cottonseed meal may have been attributed to toxicity when they were due to unrecognized nutritive deficiencies. McGowan and Crichton (*4*) have emphasized this possibility by producing typical symptoms of cottonseed-meal poisoning in pigs which received no cottonseed meal whatever, but which were fed on a deficient diet in which peanut meal was the principal component. Also Hart, Steenbock, Humphrey, and Hulce (*3*) have shown that the so-called toxicity of the oil from wheat embryo can be successfully overcome by fortifying a wheat ration with its deficiencies. Therefore, they argue, it is not "necessary to assume the presence of an inherent toxic factor in the wheat grain in explanation of the nutritive failure following the use of a ration made wholly from the wheat plant."

A comparison of the gossypol content of the meals (Table II) with their color (Table I) shows that the color of cottonseed meal can not be used as an index of the gossypol content, even though the gossypol appears to be the principal pigment in the raw seed. The color of the meal seems to be due partially to the yellow gossypol and *d*-gossypol present, and in part to a brown decomposition product which is formed from some of the constituents of the seed in the cooking process.

When this work was planned it was thought that there would be sufficient variation in the gossypol and *d*-gossypol content of the meals made by the different methods to make it possible to evaluate separately the effect of the time and the temperature of the cooking and the result of adding water to the ground seed. However, no such conclusions can be drawn because of the lack of controls, and because when all of these factors are considered collectively, even the mildest process destroys a large part of the gossypol which was probably originally present in the seed. The fact that a large part of the toxic gossypol is destroyed during the manufacture of cottonseed meal is of much importance to the livestock industry, where large amounts of meal are consumed. This has not been considered by most of the investigators in this field.

The gossypol content of raw cottonseed kernels, as determined in this laboratory, is about 0.6 per cent. Schwartze and Alsberg (*6*) give the general average of gossypol as slightly less than 1.0 per cent for this section of the Cotton Belt. Making allowance for the oil removed by the mill, the resulting meal should contain at least 1.0 per cent gossypol, provided all of the gossypol present in the seed remains unchanged in the meal. However, the largest amount of gossypol found was 0.23 per cent, only about 20 per cent of the

amount expected. The remainder has disappeared as such, or at least can not be extracted by the usual procedure. The continuous-cooker meals contained an average of 0.051 per cent gossypol, and the open-kettle meals contained a very slightly higher average amount, 0.089 per cent gossypol, thus showing that an average of 90 per cent or more of the gossypol originally present in the seed was destroyed by the process of manufacture. This statement is based on the assumption that there was no great variation in the gossypol content of the seed from which these meals were made.

It should be noted that the gossypol content of those meals which were made by the expeller method does not differ appreciably from those meals made by the continuous-cooker and open-kettle processes, in spite of the fact that the raw kernels were "cold-pressed." While Withers and Carruth (*1*, *9*) have not given any figures showing the gossypol content of expeller or "cold-pressed" meals, they have called attention to the fact that they are not made by a strictly "cold-press" method, but that the seed are heated very appreciably in the process. They have also found that in this process some of the gossypol is removed by the oil, whereas the oil from the other processes contains little gossypol.

The nature of the change of gossypol into *d*-gossypol is unknown. Withers and Carruth (*1*, *2*, *8*, *9*) thought that it is due to oxidation. On the basis of some unpublished results the writer is of the opinion that this change is due to hydrolysis. Perhaps the elucidation of this problem must await a knowledge of the chemical constitution of these two substances. At present it is not known whether *d*-gossypol is an end result of the changes which occur in gossypol during the cooking process, or whether it is a more or less stable intermediate product. It is certain, however, that there is no *d*-gossypol present in fresh cottonseed, and as the gossypol content decreases under the influence of heat and moisture the *d*-gossypol increases. The amount of *d*-gossypol present in both the open-kettle and the continuous-cooker meals indicates that the gossypol which disappears during the mill operations is converted into *d*-gossypol without any great change in molecular weight.

Under the usual mill conditions, *d*-gossypol seems to be the end product, but the writer will show, in a subsequent paper, that autoclaving cottonseed meal under certain conditions destroys most of the *d*-gossypol present or at least converts it into a form which can not be extracted in the usual manner.

The expeller meals contain noticeably less *d*-gossypol than the other types of meal. Reference has been made above to the fact that the essential features of the expeller process is the grinding of the relatively dry seed under pressure. The absence of moisture tends to prevent the gossypol from changing into the more insoluble *d*-gossypol, and at the same time the grinding and pressure favor the disruption of the resin glands which contain the gossypol and facilitate the solution of the gossypol in the oil. The net result is that a considerable part of the gossypol is removed by the oil. A part of that which is not dissolved is converted into *d*-gossypol, and a part is left unchanged in the meal.

The above discussion emphasizes the necessity for a knowledge of the amount of gossypol and *d*-gossypol present in all rations used in experiments on the toxicity or on the nutritive value of cottonseed meal.

SUMMARY

The gossypol and *d*-gossypol contents of 40 North Carolina cottonseed meals is reported. Of these 14 were made by the open-kettle method, 22 by the continuous-cooker process, and 4 by the "cold-press" or expeller process.

The gossypol content varied in the continuous-cooker meals from 0.007 to 0.228 per cent. The expeller meals contained 0.02 to 0.102 per cent, while the gossypol of the open-kettle meals ranged from 0.021 to 0.150 per cent.

The *d*-gossypol in the meals made by these processes was as follows: The continuous-cooker meals contained 0.633 to 1.076 per cent, the expeller meals 0.335 to 0.505 per cent, and the open-kettle meals from 0.544 to 0.963 per cent.

Seventy-five per cent or more of the gossypol present in the cottonseed kernels is converted, in the manufacture of cottonseed meal, into the less toxic *d*-gossypol. This indicates that this chemical compound, the constitution of which is unknown, exists in a labile condition and is readily affected by certain manufacturing conditions encountered when the oil is expressed from the seed. Unpublished data suggest that this change may be due to hydrolysis and not to oxidation.

Only 12.5 per cent of the 40 meals examined contained sufficient gossypol to cause injury in feeding experiments to the white rat when the diet is well balanced and contains not more than 50 per cent of cottonseed meal. This is based on the assumption that *d*-gossypol is nontoxic, of which there is indirect evidence.

The extent of the toxicity of *d*-gossypol is not definitely known.

LITERATURE CITED

(1) Carruth, F. E.
1917. Methods for approximating the relative toxicity of cottonseed products. Jour. Biol. Chem. 32: 87–90.

(2) ———
1918. Contribution to the chemistry of gossypol, the toxic principle of cottonseed. Jour. Amer. Chem. Soc. 40: 647–663.

(3) Hart, E. B., Steenbock, H., Humphrey, G. C., and Hulce, R. S.
1924. New observations and a reinterpretation of old observations on the nutritive value of the wheat plant. Jour. Biol. Chem. 62: 315–322, illus.

(4) McGowan, J. P., and Crichton, A.
1924. Cotton seed meal poisoning. Biochem. Jour. 18: 273–282.

(5) Schwartze, E. W., and Alsberg, C. L.
1923. Quantitative variation of gossypol and its relation to the oil content of cottonseed. Jour. Agr. Research 25: 285–295, illus.

(6) ——— and Alsberg, C. L.
1924. Relation between toxicity of cottonseed and its gossypol content. Jour. Agr. Research 28: 173–189, illus.

(7) ——— and Alsberg, C. L.
1924. Pharmacology of gossypol. Jour. Agr. Research 28: 191–198, illus.

(8) Withers, W. A., and Carruth, F. E.
1918. Gossypol, the toxic substance in cottonseed. Jour. Agr. Research 12: 83–102, illus.

(9) ——— and Carruth, F. E.
1918. Comparative toxicity of cottonseed products. Jour. Agr. Research 14: 425–452, illus.